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Ovarian polymorphism in relation to reproductive diversity and associated histological and histochemical attributes in some sporophagous tubulifera thysanoptera

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Abstract. Ovarian polymorphism involving oviparity, ovoviviparity and viviparity is discussed in relation to sporophagous species such as *Tiarothrips subramanii* (Ramk.), *Elaphrothrips denticollis* (Bagnall) and *Bacterothrips idolomorphus* Karny alongside with the comparative histology and histochemistry of polymorphic ovaries.

Keywords. Oviparity; ovoviviparity; viviparity; germarium; vitellogenic zone; pre and post vitellogenic zones.

1. Introduction

The incidence of ovoviviparity and viviparity in thrips alongside with oviparity is on record among some Phlaeothripine mycophagous species (John 1923; Bagnall 1921; Hood 1934; Viswanathan and Ananthakrishnan 1973a; Ananthakrishnan *et al* 1983b; Dhileepan and Ananthakrishnan 1984). Facultative viviparity was first reported in *Megathrips lativentris* (Heeger) by John (1923), and ovoviviparity in species of *Anactinothrips* and *Diceratothrips* species by Hood (1935). Hood (1950) showed that *Megathrips lativentris* (Heeger) and *Bactrothrips buffai* (Karny) (=*Caudothrips buffai* Karny) living in temperate regions are known to be ovoviviparous and number of other tropical species of *Diceratothrips* and *Anactinothrips* may also produce living young ones. Bournier (1951, 1956, 1957) made a detailed study on ovoviviparity common in *B. buffai* in southern France. Viswanathan and Ananthakrishnan (1973a) discussed the adaptation for survival in extreme conditions in relation to the occurrence of ovoviviparity in *Tiarothrips subramanii* (Ramk.). An analysis of reproductive strategies and behavioural aspects of some sporophagous Idolothripinae involving oviparity, ovoviviparity and viviparity in *T. subramanii*, *Elaphrothrips denticollis* (Bagnall), *Elaphrothrips procer* Schmutz were made by Ananthakrishnan *et al* (1983a). Ananthakrishnan *et al* (1983b, 1984) further described the behavioural attributes in oviposition in some of the mycophagous thrips indicated the mean fecundity rates and incubation periods of some sporophagous thrips, and showed that the oviparous forms are more fecund than the ovoviviparous and viviparous forms. Ananthakrishnan and Dhileepan (1984) also observed the functional diversity in relation to reproductive polymorphism in *Bactrothrips idolomorphus* Karny. Kiester and Strates (1984) noticed as many as 300 eggs in a colony of *Anactinothrips gustaviae* Bagnall, the eggs hatched under field conditions between 10th and 15th day after they were laid. Results herein presented relates to the histological and histochemical aspects of polymorphic ovaries and associated functional attributes.

2. Materials and methods

The sporophagous species *T. subramanii*, *B. idolomorphus*, *E. denticollis*, *E. procer* and *Adelothrips cracens* (Anan.) were collected from their respective fungus infested host plants and maintained in a BOD incubator at 27±1°C and 80% RH. Individual thrips as well as mated pairs were reared in plastic vials (4·5 × 3·0 cm) containing the specific fungal meal. Mouths of the rearing vials were covered with muslin cloth for aeration. Cotton plugs soaked in water were kept in each culturing vials for moisture, and these cotton buds were moistened periodically.

For anatomical studies, adult, pupae and larvae were dissected out in insect Ringer's solution and the testes and ovaries were fixed in Bouin's or Carnoy's fluid, dehydrated in the alcohol series, cleared in xylene, stained with Haematoxylin and Eosin and mounted in Canada Balsam. Whole-mounts of ovaries, testes and spermathecae were studied under Leitz Dialux–20 compound microscope and photographs were made using a 'wild' camera and Ilford 35 mm films (ASA 125). Numerical variations of oocytes at various stages of development both in the ovarioles as well as in the lateral oviducts were also obtained. Diagrams of the whole mounts of ovaries, testes and spermathecae were made using a camera lucida and measurements were made using a calibrated occular micrometer.

For histological studies, testes, ovaries and spermathecae were fixed in Bouin's fluid, dehydrated through the alcohol series, blocked in moulton paraffin wax and sectioned at 7 μm thickness in a rotary microtome, stained with Haematoxylin–Eosin combinations and mounted in Canada Balsam. For a study of histochemical analysis (Pearse 1968), the following tests were used: mercuric bromophenol blue test for proteins; aqueous bromophenol blue test for basic proteins; toluidine blue test for acidic proteins; Best's Carmine test for glycogen and Sudan III method for total lipids.

Mating patterns involving diverse breeding combinations between different sex-limited polymorphs were studied by freely allowing individuals to mate and the mating pairs carefully transferred to a different vial (4·0 × 3·0 cm). Without disturbing the mating process and reared separately and observations on the oviposition behaviour and fecundity were made.

3. Results

The ovaries which lie ventrolateral to the hind gut in oviparous females, extend from seventh or eighth abdominal segment to third or fourth segment, and from first or second abdominal segment and extending upto the prothorax, in view of the very long oviduct in ovoviviparous as well as viviparous forms. The lateral and common oviducts of the oviparous female is short with 4–8 fully developed oocytes laden with yolk, and never retained in the lateral oviducts, the subsequent development taking place after oviposition. Individuals with the ovoviviparous ovary have 2–4 oocytes partly laden with the yolk, developing in the long lateral oviducts only upto blastokinesis. Eggs laid in this partly developed condition hatch after a very short incubation period. All the 4 basal oocytes mature simultaneously without any yolk accumulation in the viviparous ovary. The lateral oviducts are extremely long in viviparous forms, the complete development of the pre-vitellogenic oocytes taking place in the oviducts, followed by larviposition.

The dimensions of the mature basal oocytes of the oviparous ovarioles are 3–5 and 6–10 times respectively greater than in the ovoviviparous and viviparous conditions indicating the amount of yolk deposition. The oviduct of ovoviviparous and viviparous individuals are 2–10 and 2–20 fold respectively longer than those of the oviparous individuals. Enormously long lateral oviducts facilitate the retention of the partly vitellogenic oocytes as in ovoviviparous forms till blastokinesis and the previtellogenic oocytes as in viviparous forms, till they complete their development within the lateral oviducts. The number of oocytes undergoing subsequent development in the lateral oviducts ranges from 9–11 and 5–12 in ovoviviparous and viviparous ovaries respectively.

3.1 *Histology of oviparous ovarioles*

The terminal filament, germarium, pre-vitellogenic zone with pre-vitellogenic oocytes, vitellogenic zone with vitellogenic oocytes, post-vitellogenic zone with fully mature ovum and pedicel or calyx opening into the lateral oviduct are typical of oviparous ovarioles. The post-vitellogenic zone is absent in the ovoviviparous forms with the vitellogenic zone shorter resulting in the shorter length of the ovariole. In the viviparous ovarioles only 3 regions are evident viz (i) terminal filament, (ii) germarium and (iii) pre-vitellogenic zone. Unlike oviparous ovarioles, viviparous ovarioles lack the vitellogenic and post-vitellogenic zone. As a result the pre-vitellogenic oocytes are ovulated as such, without any yolk accumulation.

The distal part of the germarium of the 4 ovarioles on each side in the oviparous individuals fuse together forming one coalescent germarium which tapers smoothly with elongated epithelial cells to form the terminal filament attached to the salivary glands. Oogonial cells of the germarium are covered by an external sheath and an internal tunica propria. Considerable differences do not occur in the number of oogonia among oviparous, ovoviviparous and viviparous ovarioles and generally the number of oogonia ranges from 8–14 in each ovariole. Each oogonium is a small spherical cell with a distinct nucleus and peripheral cytoplasm; however, the oogonia lack clear-cut demarcation from each other, these undifferentiated oogonia, mature and become fully developed by the time they reach the proximal region as they descend down the ovariole. As the oogonia descend down the pre-vitellogenic zone they have a squarish or rectangular profile with a centrally placed nucleus, occupying most of the oocyte. These pre-vitellogenic oocytes are oriented in a linear fashion and surrounded by a thin layer of follicular epithelial cells. As the pre-vitellogenic oocytes descend down, they enter the vitellogenic zone, in which continuous yolk deposition occurs and resulting in the oocytes with a solid, centrally located nucleus, increasing in dimension followed by the stretching of the follicular epithelium so as to completely cover the oocyte. It is a major zone occupying more than half the entire length of both oviparous and ovoviviparous ovarioles. The post-vitellogenic zone is characterised by the proximal, well-developed oocytes with adequate yolk reserves, each covered by an inner vitelline membrane. The interfollicular epithelial cells are very prominent in between the basal vitellogenic oocytes and the most proximal post-vitellogenic ovum. The proximal end of each ovariole opens into the distal end of the lateral oviduct through the pedicel, the lumen of which is filled by epithelial plug that is removed only during the first ovulation (figure 1).

3.2 *Histology of ovoviviparous and viviparous ovarioles*

In the ovoviviparous ovarioles, due to the absence of the post-vitellogenic zone and the relatively shorter vitellogenic zone, the ovariole length is comparatively shorter. The terminal oocytes at the proximal end of the ovarioles do not attain the full dimension as in oviparous basal oocytes indicating partial yolk accumulation. The basal oocyte is oval and externally surrounded by the follicular epithelial cells with a distinct, proximally placed nucleus.

The germarium and the pre-vitellogenic oocytes in viviparous ovarioles are almost identical to those of the oviparous and ovoviviparous ovarioles. Due to the absence of both the post-vitellogenic and vitellogenic zones, the ovarioles of viviparous ovaries are very much shorter. As the primary oogonia descend down into the pre-vitellogenic zone, they are spherical and externally covered only with ovariole sheath with a follicular epithelial covering. The pre-vitellogenic oocytes, as they descend increased moderately in their dimensions and have a large centrally placed nucleus with a very prominent compact centrally placed nucleolus (figure 2). Similarly the most proximal oocytes possess densely stained ooplasm and open into the lateral oviduct through a short pedicel. Since both vitellogenic and post-vitellogenic zones which form two-thirds of the entire length of the oviparous ovarioles are lacking, the viviparous ovarioles are 2–3 times shorter than the oviparous ones. The pre-vitellogenic oocytes without any traces of yolk ovulate into the lateral oviducts, where the subsequent embryogenesis takes place during which time they derive nutrients from maternal resource.

3.3 *Numerical variations of oocytes in polymorphic ovaries*

Considerable variations occur in the number of vitellogenic oocytes that are in the process of accumulating yolk in the ovarioles, post-vitellogenic oocytes (the most basal ovum fully laden with yolk in the ovariole), and the number of oocytes or embryos undergoing subsequent development in the lateral oviducts.

Studies on 5 species of sporophagous Tubulifera viz, *B. idolomorphus*, *T. subramanii*, *E. procer*, *E. denticollis* and *Ethirothrips agasthya* (Bagnall) indicate that the number of oogonial cells and the pre-vitellogenic oocytes does not differ among polymorphic ovaries, ranging from 46–59 and from 11–17 in each ovary. However, the number of vitellogenic oocytes in each ovary is higher (10–12/ovary) in oviparous forms, comparatively less (6–9/ovary) in ovoviviparous forms and are lacking in the viviparous ovaries. Mature ova are evident only in the oviparous forms (3–4/ovary). The number of embryos undergoing development in each lateral oviduct is higher in viviparous ovaries (10–12/each lateral oviduct) than in ovoviviparous ovaries (6–10/each lateral oviduct). A positive correlation is evident between the number of oocytes/embryos developing in the lateral oviduct and the length of the lateral oviduct, in which the latter increases proportionately with increase in the number of developing embryos.

3.4 *Histology of oviducts*

Histological studies of both lateral and common oviducts of *T. subramanii* of viviparous ovaries, revealed the presence of single layer of secretory columnar

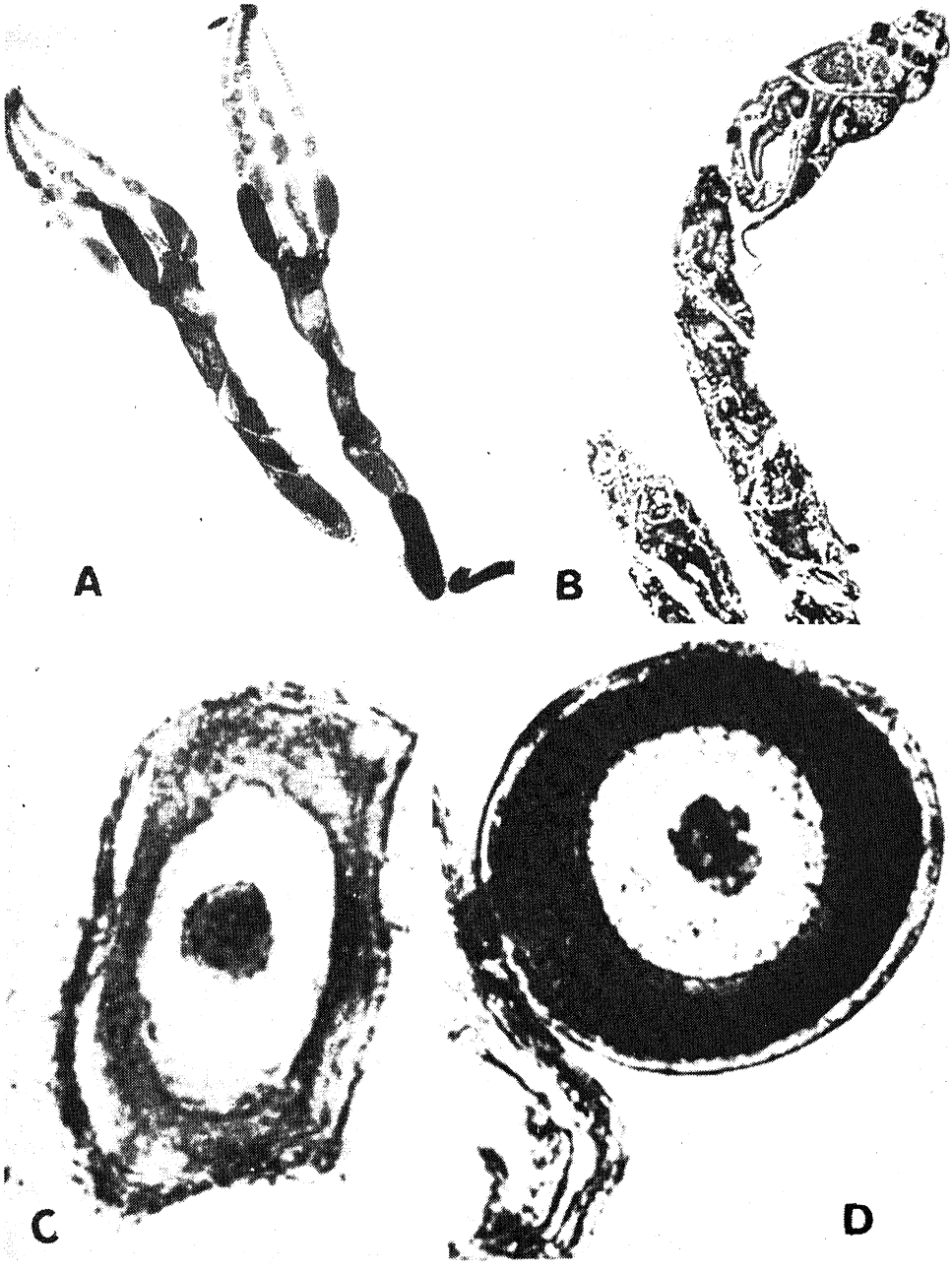

Figure 1. **A.** Oviparous ovary of Bactrothrips idolomorphus (× 35). **B.** L. S. of lateral oviduct showing developing embryos (× 125). **C.** Pre-vitellogenic oocytes (× 1000). **D.** Spherical basal pre-vitellogenic oocytes (× 780).

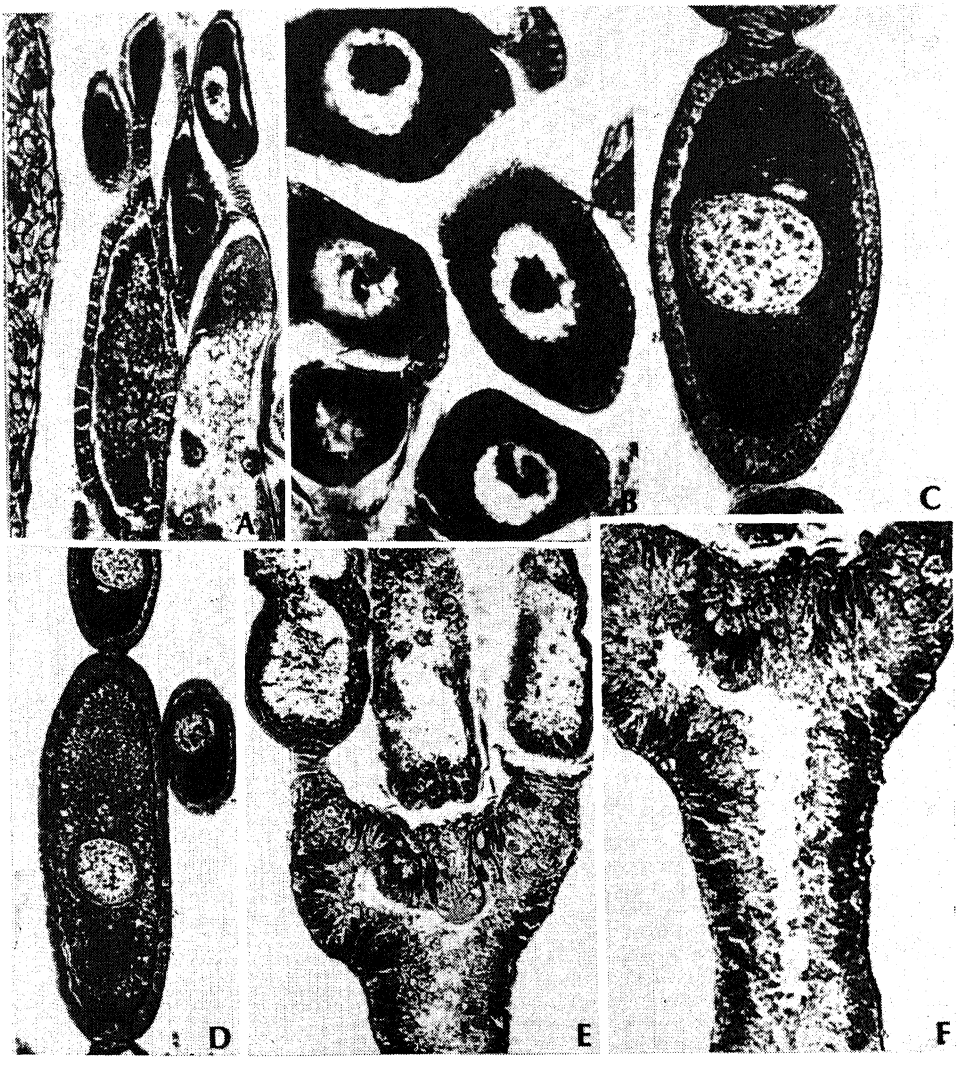

Figure 2. Histology of oviparous ovary of *T. subramanii*. **A.** L. S. of the oviparous ovary (× 300). **B.** Pre-vitellogenic oocytes (× 1250). **C.** Early vitellogenic oocytes (× 600). **D.** Vitellogenic oocytes (× 600). **E.** L. S. of lateral oviduct (× 300). **F.** L. S. of common oviduct (× 360).

epithelial cells resting on a basement membrane. These secretory epithelial cells are apocrine in nature with a very large centrally placed nucleus and a prominent, but eccentrically placed nucleus. Varying number of vacuoles are evident near the basement membrane. At the distal free margins of the secretory cells, a large number of secretory vesicles occur. The presence of secretory granules, dense cytoplasm and numerous vacuoles suggest the secretory nature of the epithelial cells.

The lateral oviducts of ovoviviparous ovaries exhibit secretory cells only at the proximal one-third of the lateral oviducts, while the rest of the distal two-thirds region lack secretory cells. In the lateral oviducts of the ovoviviparous ovaries, the

secretory regions at the proximal end of the oviducts are highly variable in length, depending upon the number of oocytes/embryos undergoing development in these oviducts, as well as on the degree of the ovoviviparity, especially the gestation period of the embryos and the stage at which the embryos are laid. In ovoviviparous oviducts the secretory cells lining the proximal region of the lateral oviduct and common oviduct are tall, columnar with centrally placed nucleus and are apocrine. The secretory nature of the cells are evident only in the lateral oviducts where the embryos are in an advanced stage of development. In those lateral oviducts with embryos in the early stage of development, the cells are inactive with dense cytoplasm and without any secretory granules. With the ovulation of the mature ovum with partly deposited yolk, the subsequent development of the oocyte takes place in the distal region of the lateral oviducts which are devoid of any secretory cells. In the viviparous ovaries both the lateral and common oviducts do not have any secretory cells and consist only of the basement membrane.

3.5 *Histochemistry of polymorphic ovaries*

Histochemical studies involving total proteins, acidic proteins, basic proteins, glycogen and lipids indicated the diversity in yolk deposition in polymorphic ovaries. Pre-vitellogenic oocytes in oviparous, ovoviviparous and viviparous ovaries show a protein positive reaction, where acidic proteins are abundant in the nucleus and perinuclear cytoplasm. Pre-vitellogenic oocytes of all the 3 types of ovaries showed a negative reaction to the glycogen and a weak lipid reaction. In the vitellogenic oocytes of both oviparous and ovoviviparous forms the intensity of protein and lipids increase both in nucleus and in ooplasm (figures 3 and 4).

Histochemical observations of the yolk granules of polymorphic ovaries indicate the incidence of increased total proteins as well as both basic and acidic protein yolk granules evenly dispersed in the ooplasm of the vitellogenic and post-vitellogenic oocytes. In the oviparous ovarioles the protein yolk granules, especially the acidic ones are more dense than the basic protein granules. In addition, both glycogen and lipid yolk granules are evenly distributed. In the vitellogenic oocytes of ovoviviparous ovarioles the glycogen granules are more abundant than in the oviparous vitellogenic oocytes. Moreover the ooplasm also has protein and lipid yolk evenly distributed in the peripheral ooplasm. In viviparous ovarioles with only pre-vitellogenic oocytes no yolk granules were evident, indicating the absence of yolk deposition.

3.6 *Embryogenesis in polymorphic ovaries*

In oviparous ovaries the fully mature eggs with yolk reserves are laid with a protective chorion and the embryogenesis takes place only after they are laid. In the ovoviviparous ovaries, mature oocytes in partly yolk-accumulated condition are ovulated into the lateral oviduct, where embryogenesis continues upto blastokinesis. There is a positive correlation between the increase in the size of the embryos in the lateral oviducts and the distance traversed by the embryos in the lateral oviducts. This correlation suggests that there is a quantitative increase in the size of the embryo as it descends down the lateral oviducts, indirectly indicating the nutrient

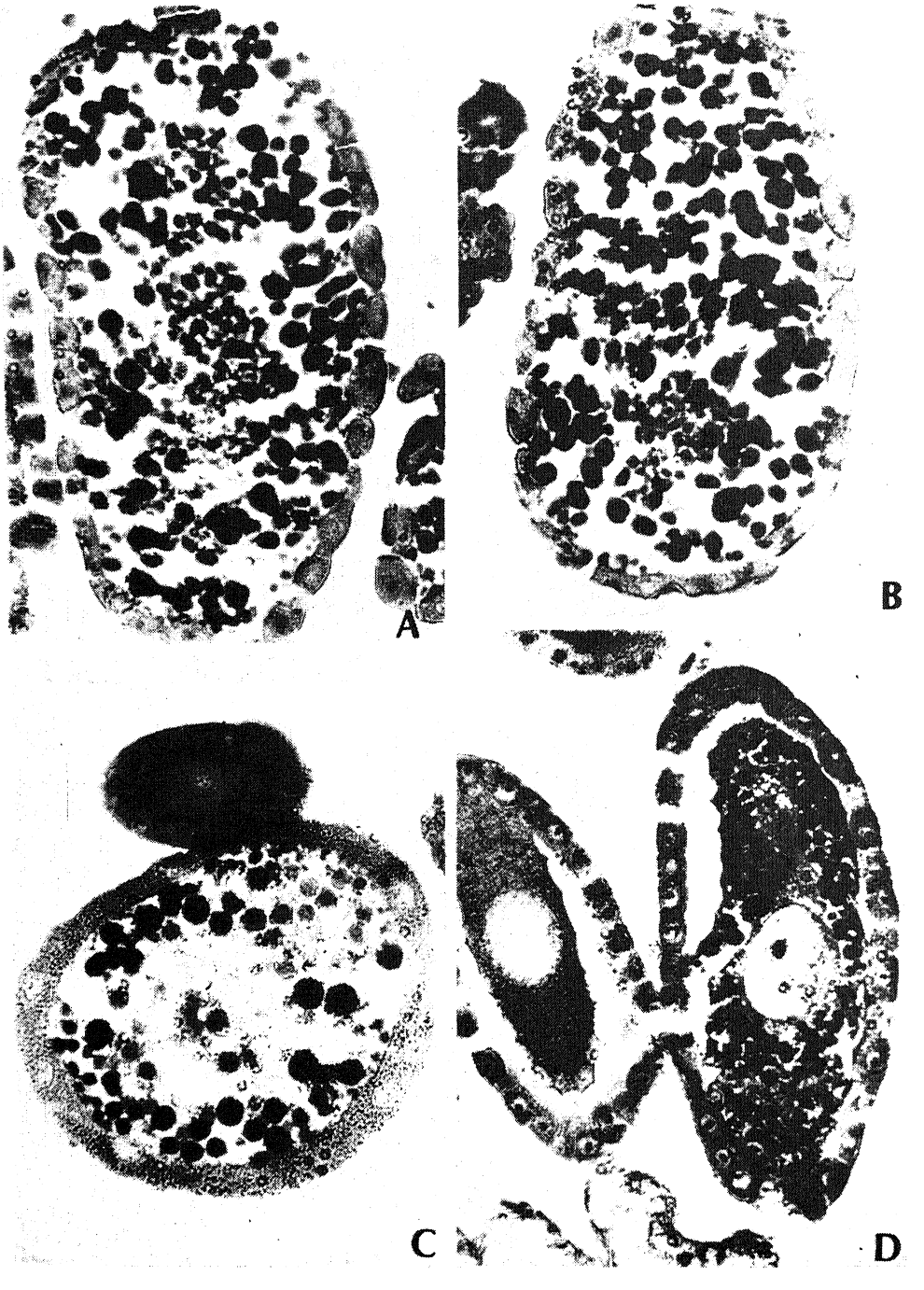

Figure 3. Histochemistry of polymorphic ovaries of *T. subramanii*. **A.** L. S. of oviparous basal oocyte showing positive reaction to total proteins (× 400). **B.** Dense acidic protein evenly distributed in the ooplasm of oviparous basal oocytes (× 400). **C.** Oviparous basal oocyte showing basic protein granules (× 350). **D.** Ovoviviparous basal oocyte showing basic protein positive reaction (× 400).

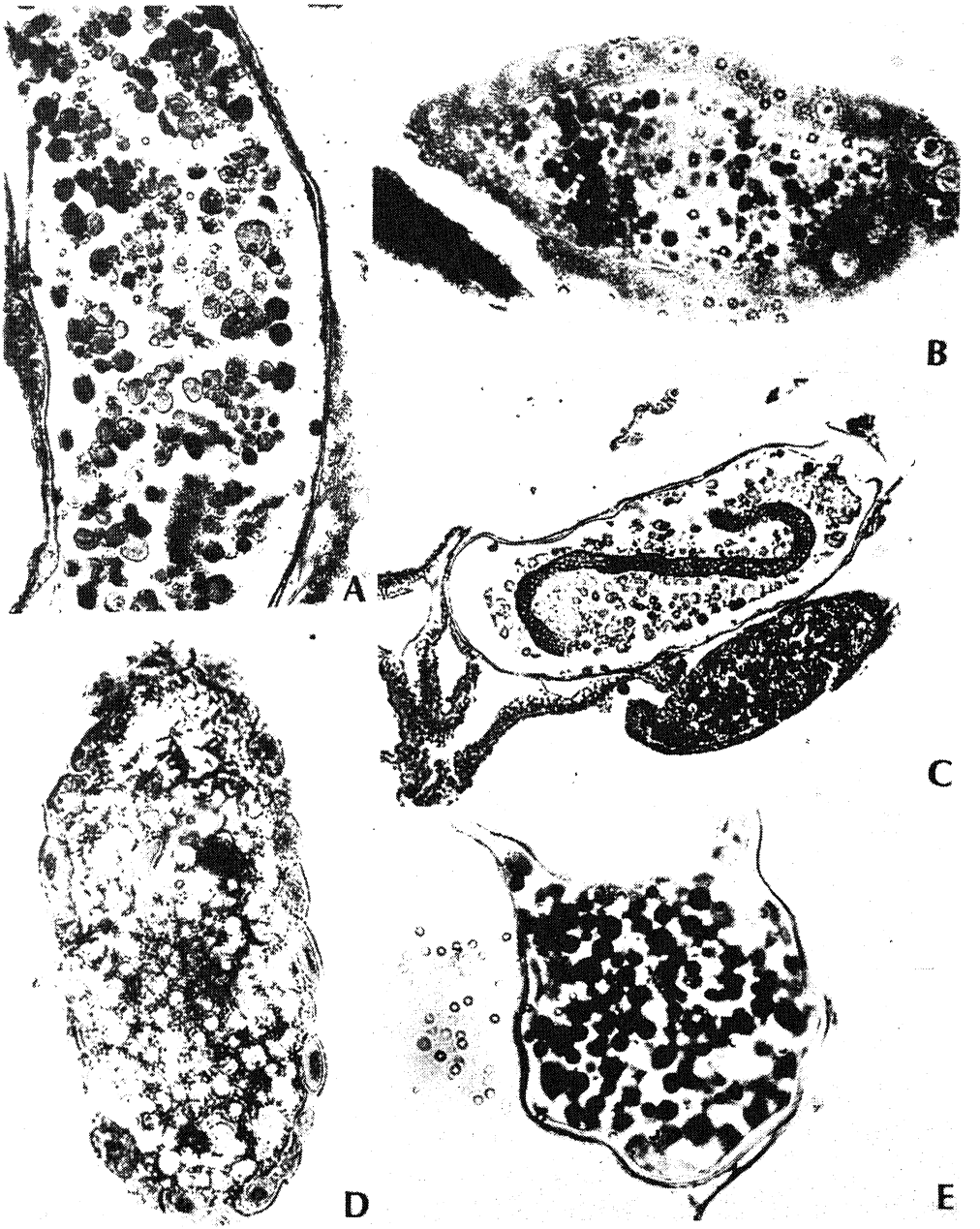

Figure 4. Histochemistry of polymorphic ovaries of *T. subramanii*. **A.** Oviparous basal oocyte showing glycogen spheres (× 650). **B.** Ovoviviparous basal oocyte showing acidic protein positive reaction (× 400). **C.** L. S. of ovoviviparous embryo in the lateral oviduct with glycogen granules (× 300). **D.** Ovoviviparous basal oocyte with lipid yolk granules (× 350). **E.** Oviparous basal oocyte showing protein yolk granules (× 350).

intake from maternal resource. The remaining embryonic development takes place after they are laid. In viviparous ovaries the pre-vitellogenic oocytes without any yolk reserves are ovulated into the lateral oviduct where the complete embryonic development takes place and fully developed larvae are laid as such. Histology of the lateral oviducts with developing embryos whose stage of development increased towards the distal most region of the lateral oviduct which opens into the common oviducts. Embryos at the proximal end of the lateral oviduct are only at initial stage of embryogenesis and smaller in size. As they move down, there are large number of embryos at various developmental stages. Those at the distal end of the lateral oviduct or in the common oviducts are in the stage of completing the embryogenesis or as fully formed larvae. Since the lateral oviducts of the viviparous ovaries contain many embryos, histological studies revealed all the stages of embryonic development in a sequence. Further there is a significant, proportionate increase in the size of the embryos as they descend down in the lateral oviduct.

4. Discussion

Both the ovoviviparous and viviparous ovaries differ from each other, as well as from oviparous ovaries in structure and function. Statistical analysis of 5 species of diversely reproducing idolothripines like *B. idolomorphus*, *T. subramanii*, *E. procer*, *E. denticollis* and *M. menoni* indicated a significant reduction in the ovarioles of both ovoviviparous and viviparous forms with corresponding elongation of the lateral oviducts. The oviparous ovarioles are longer due to long vitellogenic zone with post-vitellogenic zone containing larger basal oocytes. The viviparous ovarioles are shorter due to the absence of vitellarium. However, in ovoviviparous ovarioles the vitellarium is short with partly developed basal oocytes, resulting in shorter size. Since the vitellarium occupies 2/3 of the entire ovariole, their absence or reduction significantly alters the ovariole length. Similar reduction of vitellarium was also reported in ovoviviparous and viviparous Blattaria (Roth 1964).

The enormously long lateral oviducts facilitate the retention and nourishment of the embryos. The elongation is pronounced as early as the pupal stage when ovulation has not yet commenced. This increased length of the lateral oviducts is due to continuous ovulation and retention of oocytes/embryos. This is supported by the positive correlation between the increase in the lateral oviduct length and increase in the number of embryos in the lateral oviduct. Bournier (1957, 1962, 1966) noticed the retention of eggs in the long lateral oviducts of ovoviviparous *B. buffai*, but their structural adaptations in relation to egg retention were not revealed. Similar long lateral oviducts retaining embryos were also reported in ovoviviparous *T. subramanii* (Viswanathan and Ananthakrishnan 1973) and *B. brevitubus* (Haga 1975).

As a result of structural variations among polymorphic ovaries their relative position in the females were also considerably altered. In both ovoviviparous and viviparous forms the elongated lateral oviducts resulted in the shifting of the ovarioles towards the anterior side of the abdomen, near the metathorax. In oviparous ovaries, due to the short lateral oviducts, the long ovarioles emerged from the posterior portion of the abdomen and extended upto metathorax. Haga (1975) has also mentioned about the relative position of ovaries of the oviparous and ovoviviparous *B. brevitubus*.

Both ovoviviparous basal oocytes with partial yolk deposition and viviparous basal oocytes without any yolk are not covered with chorion, before ovulation. John (1923) also reported in *M. lativentris* the absence of chorion when larvae were laid and the presence of a distinct chorion when the insects laid eggs. A similar phenomenon is also observed in the polyctenid *Hesperoctenes fumarius* Westwood and several other viviparous species (Hagan 1951). The most important role of follicular epithelium during insect oogenesis has been considered to be synthesis and deposition of egg envelope during choriogenesis (Mahowald 1972). However, in the gall midge *Heteropeza pygmaea* Winnertz, which exhibits paedogenesis, the chorion is not formed at the end of oogenesis and the growing embryos remain enveloped by the folliculae epithelium (Junquera 1983). Hansen (1894) and Heymons (1909, 1912) noticed that the eggs of viviparous *Hemimerus taploides* Walker has no chorion and contains little yolk and the thickened follicular epithelium provides nutrients for the growing embryo.

4.1 *Numerical variation of oocytes in polymorphic ovaries*

In addition to anatomical and histological variations, numerical variation of oocytes and embryos in the polymorphic ovaries also occur. The higher number of vitellogenic and post-vitellogenic oocytes in oviparous forms and comparatively lesser number of only vitellogenic oocytes in ovoviviparous forms appear to be due to the long vitellarium as in oviparous forms and shorter in ovoviviparous species. Incidence of post-vitellogenic ovum only in oviparous forms suggests that complete maturation of the ovum occurs only in oviparous forms. Correspondingly the number of embryos in the lateral oviducts of the viviparous ovaries are higher than in ovoviviparous ovaries. This is because of the prolonged gestation period of the viviparous embryos than the ovoviviparous embryos. Haga (1974) reported as many as 24 embryos, 11 in the right and 13 in the left lateral oviducts of *B. brevitubus*. As more number of pre-vitellogenic oocytes undergo vitellogenesis to become vitellogenic oocytes, the number of pre-vitellogenic oocytes are retained as such resulting in their increased numbers. Moreover the frequency of ovulation of pre-vitellogenic oocytes is low in viviparous forms, as compared to the ovulation of mature ova and partly developed ova in oviparous and ovoviviparous ovaries respectively.

4.2 *Histochemistry of polymorphic ovaries*

Histochemical analysis revealed no yolk granules in basal oocytes of viviparous ovarioles indicating the absence of vitellogenesis. On the contrary in both ovoviviparous and oviparous ovaries, protein, lipid and glycogen yolk granules were evident substantiating the occurrence of vitellogenesis. Bonhag (1958) reported the incidence of protein, lipid and glycogen particles as the main deutoplasmic substances of oocytes, where the protein yolk are numerous while the glycogen may not be present in all insect eggs. The intensity of these yolk granules was dense in oviparous forms, and less dense in ovoviviparous basal oocytes. Though the incidence of glycogen yolk granules is not very common among insects (Bonhag 1958), present observations indicate the presence of glycogen granules in both

oviparous and ovoviviparous oocytes, their intensity being higher in the latter. Instances of glycogen deposition in eggs during vitellogenesis are reported in Anoplura (Ries 1932), Bumble bees and *Anisolabis* sp. (Bonhag 1958).

Structural and functional modifications involving the histology of the lateral and common oviducts, to suit the type of reproduction, vary among polymorphic ovaries. In both complete ovoviviparous and viviparous ovaries the lateral oviduct wall is without any secretory cells and are stretched into a thin membrane. The developing embryos without chorionic covering lie close to the wall of the lateral oviducts and derive nutrients through thin membranous part. Haga (1975) also reported similar thin and transparent lateral and common oviduct walls in ovoviviparous *B. brevitubus*. Thus, the absence of secretory cells facilitates the embryonic nourishment through the lateral oviduct walls. However, in partial ovoviviparous ovaries, the secretory cells at the distal end of the lateral oviducts alone are concerned with the embryonic nourishment, whereas those at the proximal secretory region are responsible for the chorion secretion, for the eggs that are laid. Thus the length of the secretory region of the oviduct is inversely proportional to the stage of the embryonic development at the time of laying. Greater the secretory region, earlier is the stage of the embryo laid, and when the secretory cells are absent, fully developed larvae are laid. Though in general, the chorion is secreted by the follicular epithelium in the ovariole (Mahowald 1972), in both ovoviviparous and viviparous ovaries presently observed, the oocytes that are ovulated are without chorion. Since only embryonated eggs are laid in ovoviviparous forms, it is assumed that the chorion might be secreted by the cells lining the lateral and common oviducts. However, in viviparous forms showing direct larviposition secretory cells are absent.

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Host preferences of some acridids (Insecta: Orthoptera) in relation to some biochemical parameters

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Abstract. Biochemical analysis of host plants of 6 species of acridids for their total protein, carbohydrates, phenols, free aminoacids, water and nitrogen content revealed significant correlation in regard to their host preferences, wherein the leaf water-nitrogen index as well as the total phenol composition appeared to play an important role.

Keywords. Biochemical parameters; host preferences; acridids.

1. Introduction

The presence or absence of phagostimulants/deterrents in the right proportion to facilitate optimal food utilization is of significance in host selection in acridids (Mulkern 1967). Less preferred/non-host plants exhibit varied resistance mechanisms in terms of such chemical parameters as their total nitrogen, protein, aminoacids and phenols (Chapman and Bernays 1977; Miller and Strickler 1984). Specialist and generalist feeders are equally common among acridids, some exclusively graminaceous feeders, others only on dicots and yet others on both (mixed feeders). In terms of the quantitative intake of leaves which influences the biotic potential of the insect (Ananthakrishnan *et al* 1985a, b), an analysis of the chemical factors of the host plants appear relevant for a basic understanding of the nature of host preferences of such acridids as *Oxya nitidula* (Walker) (Oxyinae), *Truxalis indica* Boliver (Truxallnae), *Orthacris maindroni* Boliver (Pyrgomorphidae), *Atractomorpha crenulata* (Fabricius) (Pyrgomorphidae), *Aiolopus thalassinus* (Fabricius) (Oedipodinae) and *Cyrtacanthacris ranacea* Stoll (Cyrtacanthacridinae).

2. Materials and methods

Different species of acridids were collected from the fields of *Oryza sativa*, *Panicum maximum* and *Gossypium hirsutum* and mass reared in the laboratory on the various host plants in wooden cages measuring 25 × 25 × 30 cm. The duration of development of the acridids on the various host plants was observed and this was taken as an index for the host preferences of the species.

The host leaves were subjected to biochemical estimation for the total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1965), nitrogen (Vogel 1963), phenol (Hori 1974) and total free amino acids (Moore and Stein 1948). The moisture content was estimated by weighing accurately 2 g of the sample and incubating at 37°C till a constant weight was obtained. The difference in weight was calculated and the moisture content represented as percentage.

3. Results

Food preference in terms of the rate of development on various host plants indicated faster development when reared on the preferred host, the quickest rate being that of *A. crenulata* when fed on *Ricinus communis* (37·7 ± 5·9 days), and the slowest when fed on *Solanum torvum* (99·4 ± 10·7 days). In the case of *O. maindroni* the longest nymphal duration was observed when fed on *Clerodendron* sp and shortest on *Dolichos lablab*. Faster post embryonic development of *C. ranacea* occurred when fed on *Gossypium hirsutum* (50·0 ± 0·94 days) as against the malvaceous weed *Abutilon indicum* (62·0 ± 1·66 days). *Panicum maximum* appeared to facilitate faster nymphal development of *Aiolopus thalassinus* and *T. indica* and *Cyperus rotundus* for *O. nitidula* (Ananthakrishnan *et al* 1985a,b).

O. nitidula feeds on poaceous and cyperaceous weeds and graminaceous crops avoiding dicotyledons. Likewise, *Truxalis indica* and *A. thalassinus* feed exclusively on graminaceous plants, whereas *A. crenulata* is a mixed feeder, feeding both on monocots and dicots. *C. ranacea* was observed to have a comparatively narrow feeding range, specifically confined to feeding on malvaceous plants thereby earning the 'specialist' status. Individuals of *O. maindroni* feed on a wide spectrum of dicots exhibiting greater preference towards *R. communis* and to a lesser extent on *D. lablab*.

Quantitative biochemical analysis of the leaves of the host plants in terms of water, nitrogen, proteins, carbohydrates, aminoacids, phenols and silica content revealed striking correlation with the food preference of these acridid species (table 1). Acridids feeding exclusively on monocots generally preferred host with low water content with the exception of *A. thalassinus*. The dicot as well as mixed feeders preferred hosts with high water content. An estimation of the water content of the host plants of *O. nitidula* indicated that *P. maximum*, one of the natural hosts with higher water content (77·29%) to be the least preferred. Though the water content of *C. rotundus* was less than that of *P. maximum*, the leaves of the former were readily consumed by all the stages of *O. nitidula*. Although the percentage of water in *O. sativa* (60·77%) and *C. dactylon* (61·97%) was nearly the same, *C. dactylon* was preferred to *O. sativa*, presumably due to the effect of the other chemical stimulants of the host. Similarly for *T. indica*, the most preferred host *C. dactylon* had a lesser water content. *A. crenulata* and *O. maindroni* preferred *D. lablab* with a higher water content (83%). For the specialist, *C. ranacea*, *Abelmoschus esculentus* with a higher water content (80·49%) was preferred less to *G. hirsutum*. Although the susceptibility of the host to insect attack is known to depend on the nitrogen content of the plant, the water-nitrogen ratio provided a better insight of the resistance of plants to acridids, showing a distinct correlation of their relative preference with the water-nitrogen index of the host, preferring the host with a low index. However, the mixed feeder, *A. crenulata* and *T. indica* preferred hosts with a high water-nitrogen index (figure 1).

As the total free amino acid content of the monocots is quantitatively lower than the dicots, an analysis of the comparative feeding preferences of the acridids indicated a preference for plants with higher amino acid content. The monocot feeding acridids generally showed a greater preference for feeding on higher amino acid content plants. All the acridids studied here appeared to show greater preference to leaves with high carbohydrate content, proteins being generally known to be essential for the growth and development of the insect. A comparative assessment of

Table 1. Chemical composition of different acridid host leaves.

Host	Proteins (mg/g)	Carbohydrates (mg/g)	Phenols (mg/g)	Amino acids (mg/g)	Nitrogen (%)	Water (%)	Water/N_2
1. *Cyperus rotundus*	42·00	193·0	73·5	0·190	6·72	71·74	10·67
2. *Cynodon dactylon*	55·50	586·0	102·0	0·124	8·88	61·97	6·97
3. *Panicum maximum*	159·00	586·0	160·5	0·140	25·44	77·29	3·04
4. *Oryza sativa*	63·00	248·0	106·5	0·102	10·08	60·77	6·03
5. *Gossypium hirsutum*	55·00	440·0	232·5	0·152	8·80	73·16	8·31
6. *Abelmoschus esculentus*	12·50	515·0	245·0	0·240	2·00	80·49	40·24
7. *Abutilon indicum*	35·00	407·5	165·0	0·220	5·60	69·50	12·41
8. *Sida rhomboidea*	97·50	302·5	245·0	0·092	15·60	46·36	2·97
9. *Ricinus communis*	68·00	200·0	38·0	0·464	10·88	75·00	6·89
10. *Arachis hypogaea*	92·39	17·0	24·0	0·365	14·78	72·00	4·87
11. *Solanum torvum*	163·04	44·0	20·5	0·256	26·08	77·00	2·95
12. *Calotropis gigantea*	259·33	12·5	22·0	0·120	41·49	88·00	2·12
13. *Dolichos lablab*	189·53	275·0	55·0	0·345	31·76	83·00	2·61
14. *Clerodendron* sp.	80·00	425·0	85·0	0·158	12·80	80·00	6·25

1–4, Hosts of *O. nitidula*; 5–8. Hosts of *C. ranacea*; 1–3. Hosts of *T. indica*; 1–3. Hosts of *A. thalassinus*; 3. 9–11. Hosts of *A. crenulata*; 9, 12–14, Hosts of *O. maindroni*.

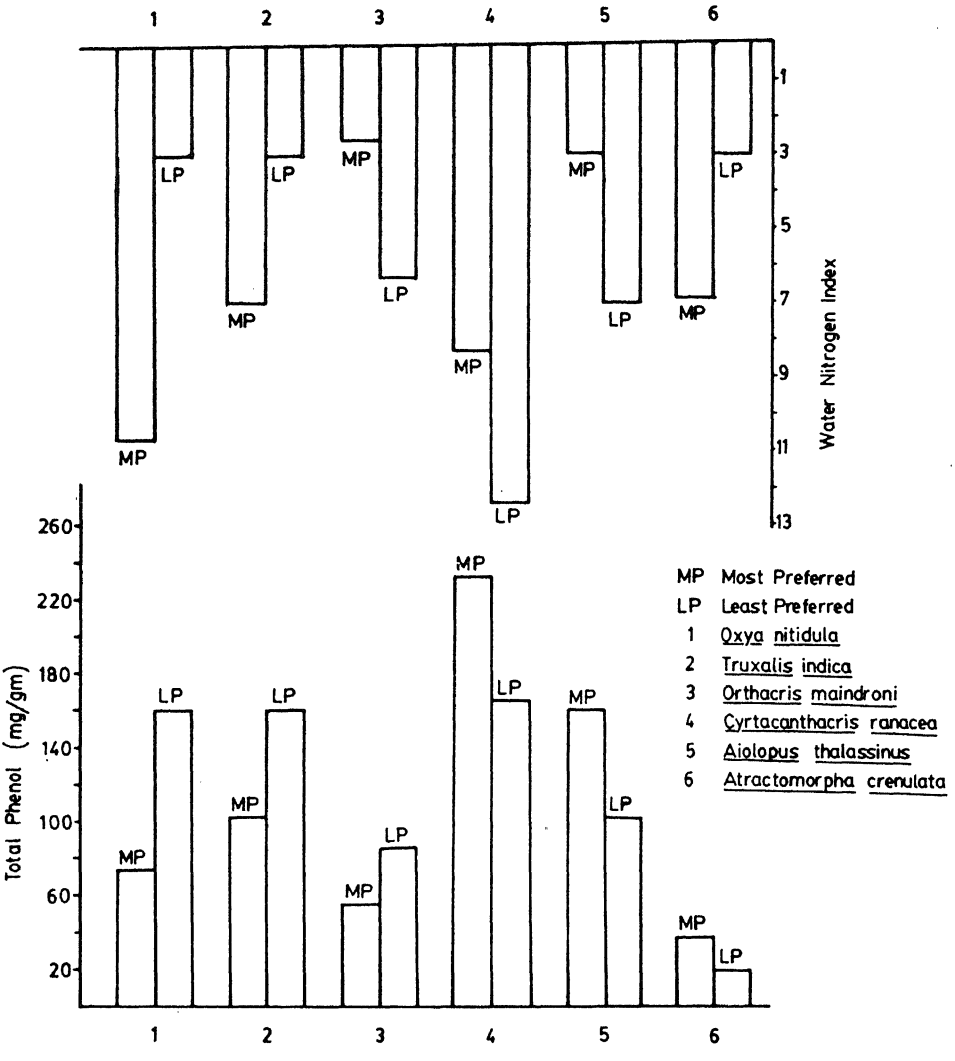

Figure 1. Comparative preference of different acridid species in relation to total phenols and water-nitrogen index of host plants.

the host plants for the total protein content indicated that the mixed as well as dicot feeders preferred hosts with high proteins, even though the monocots feeders were capable of thriving on plants with low protein content. The phenolic compounds are considered to act as deterrents to feeding in grasshoppers is evident in that the most preferred host plants of *O. nitidula*, *A. crenulata* and *O. maindroni* seemed to have a lesser phenol content as compared with the lesser preferred hosts (figure 1). *G. hirsutum*, the preferred host of *C. ranacea* has a less phenol content (232·5 mg/g) in comparison to *A. esculentus* (245·0 mg/g). However, a reverse trend was observed in the case of *A. thalassinus* and *T. indica*, which preferred hosts with higher phenol

content and this is presumably be due to their capacity to convert phenols into phenylalanine as proposed for *Schistocerca gregaria* by Bernays and Woodhead (1982).

4. Discussion

The choice of plants by the acridids seem to reveal that these may be based on the absence of feeding deterrents or their presence in smaller amounts in the host plants. For instance *O. nitidula* totally rejected all forbes, perhaps due to the presence of one or more compound deterrent to the graminivore (Bernays and Chapman 1977). General selection of food by an adult acridid appears to have a greater chemical basis (Bernays and Chapman 1970). *O. nitidula*, *T. indica* and *A. thalassinus* are found to select and feed constantly on the monocots only. This is possibly due to the favourable sensory inputs from the sensilla of the mouth parts and preoral cavity (Haskell and Schoonhoven 1969). Though the feeding activity could be initiated by the sensory receptors of the insect, the activation of these receptors requires a stimulatory factor to evoke normal feeding behaviour. The leaf surface chemicals play an important role in determining the feeding activity and acridids have been known to identify the phagostimulatory ones from the deterrents by palpation and recognise the leaf form from the leaf surface attributes (Chapman 1977).

Some hosts are rejected because of the presence of chemicals deterrent to feeding and such deterrent chemicals seem to play an important role in the feeding behaviour of acridids. Further, some secondary compounds that are deterrent for *Locusta* are ineffective for *Schistocerca* and some which are deterrent at high concentrations stimulate feeding at lower concentrations (Bernays and Chapman 1978). The substances which may stimulate feeding are specific and the occurrence of suitable phagostimulants may, in part, effect feeding. Some amino acids (proline and serine), hexose sugar and disaccharides are found to be phagostimulatory for *L. migratoria* (Cook 1977). Acridid species have evolved a diversity to recognise only small number of phagostimulants rather than a large number of feeding inhibitors. Hence feeding depends on the balance between the phagostimulants and the feeding deterrents and the response varies in different species so that a plant may be acceptable to some, but not to other species, or the rejection of hosts by these insects may be owing to the presence of one or more chemicals in amounts which inhibit feeding; some host plants are accepted only because of the absence of deterrent chemicals in sufficient quantities to limit feeding (Bernays and Chapman 1977; Bernays 1978).

Acridids are generally observed to take larger meal on lush grass with high water content (Chapman and Bernays 1977). Although Bernays and Chapman (1970) felt that moisture content may not play any part in the food selection of *Chortippus parallelus*, it cannot be ignored in the acridids studied here as the food plant provide water in excess of the requirements of the insect. Scriber (1984) suggested that the post ingestional growth performance in phytophagous insects is determined to a large extent by plant chemical quality and can be indexed by leaf water-nitrogen composition. All the acridids studied here appeared to show greater preference for host plants with low water-nitrogen index.

Total phenol content of the host plant appears to interfere with the food selection of the acridids. The high phenol content makes *D. lablab*, the least palatable, while

R. communis with least phenol content is most preferred for *A. crenulata*. The feeding preferences of *C. ranacea* only attest the fact that food selection depends not so much on specific phagostimulants in the acceptable plants as in the presence of inhibitory chemicals in the non-acceptable plants (Thorsteinson 1960). A very high concentration of sugar usually limits the amount of food taken (Cook 1977). However, *A. crenulata*, *O. maindroni* and *T. indica* appear to show greater preference for host with high sugar content. Hence, food selection of these acridids presumably involves, besides many physical and chemical factors, the balance between deterrent chemical and phagostimulants (Chapman and Bernays 1977), the inhibitory factors very often overriding the stimulatory ones.

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Ecobehavioural and biological studies on two species of seed infesting thrips (Thysanoptera : Insecta)

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Abstract. Ecobehavioural and biological aspects of two species of seed infesting thrips, viz *Chirothrips mexicanus* Crawford and *Chiridothrips indicus* Ramk. and Marg. are discussed with particular reference to their reproductive biology, population dynamics on different host plants and their damage potential to seed production of their primary host plants.

Keywords. Seed-infesting thrips; *Chirothrips mexicanus*; *Chiridothrips indicus*; damage potential.

1. Introduction

Loss in seed production due to thrips infestation on graminaceous plants is attributed to *Chirothrips hamatus* Trybom (Vappula 1965; Hukkinen 1936), *Chirothrips mexicanus* Crawford, (Roney 1949; Riherd 1954; Ananthakrishnan and Thirumalai 1977, 1978), *C. pallidicornis* Priesner (Doull 1956) and *C. falsus* Priesner (Watts 1965). *Frankliniella* spp. (Bailey 1948) and *Kakothrips robustus* (Uzel) (Bhul 1973; Franssen 1960) are also known to reduce seed production in some dicots. Being seed infesting species, both *C. mexicanus* and *Chiridothrips indicus* Ramk. and Marg. considerably reduce seed production, controlling the propagation of the weed host. Results presented here attempt to discuss the reproductive biology of the species, their population trends on different host plants and their damage on seed production.

2. Material and methods

The inflorescence of *Chloris barbata* Sw., *Eleusine aegyptiaca* Desf. and *Eleusine indica* Gaertn. along with the adults and immature stages of *C. mexicanus*, were collected in plastic bags and subsequently transferred to a collecting chamber comprising a chimney with a split cork at the bottom and with a polythene bag fitted with a rubber band at the top. The flower stalks were inserted through the split cork and the cut ends were kept in water in a wide mouthed bottle. The emerging adults that always moved upwards, were collected from the polythene bag using a camel hair brush (No. 0).

The inflorescence of *Achyranthes aspera* L. along with the varying stages of *C. indicus*, a seed infesting tubuliferan were brought to the laboratory in a tightly closed polythene bag. After gentle tapping on to a white pad, the thrips were transferred to a vial of 70% alcohol with a camel hair brush. For biological studies, the methods followed by Ananthakrishnan and Daniel (1981) were adopted. For population studies of *C. indicus* the dry count technique of delayed counting method as well as direct counting method (Irwin *et al* 1979) and for *C. mexicanus* the common

beating technique (Ananthakrishnan 1969) were followed. Population studies were carried out twice a month for a period of 3 years. For assessing the parthenogenetic reproduction of *C. mexicanus*, the male prepupae were removed and the female prepupae cultured on the 3 different host plants. In *C. indicus* mating was observed by releasing males and females in solidified, paraffin-filled petriplates with a shallow moat (Varadarasan 1979). In order to establish a possible relationship between the number of ovaries damaged by feeding and oviposition, the correlation coefficient '*r*' was computed.

3. Observations

3.1 *Sexual dimorphism and parthenogenesis*

Females of *C. mexicanus* are macropterous with an average life span of 17·2 days and reproduce both sexually and parthenogenetically. The smaller apterous males exhibit an average life span of 7·3 days. In *C. indicus* that reproduce only sexually, both the sexes are macropterous, and do not exhibit variation in their life span of 15–20 days. The number of eggs laid by *C. mexicanus* vary with different host plants, fewer eggs resulting from parthenogenesis. On *C. barbata*, the parthenogenetic females lay on an average 26·0 ± 3·62 eggs throughout their life span, the fecundity index (number of eggs laid/preoviposition period) being 7·77 ± 1·64. On *E. aegyptiaca* and *E. indica* an individual female produces an average of 29·5 ± 4·89 and 14·92 ± 3·09 eggs, the fecundity index being 8·39 ± 2·47 and 3·59 ± 1·02 respectively (table 1). The preoviposition period in parthenogenetically reproducing individuals also varies on different host plants.

3.2 *Mating, oviposition and fecundity*

Mating of adult males with premature females (pupae) is noticed in *C. mexicanus* (Ananthakrishnan and Daniel 1981) without subsequent mating after metamorphosis into adults. Males are polygamic and resort to multiple mating with several female pupae. After mating for 1–3 min the males move to new spikelet in search of another

Table 1. Comparison of sexual and parthenogenetic reproduction of *C. mexicanus* on different host plants.

Host plant		Life span of adults (days)	Preoviposition period (days)	Fecundity[a]	Fecundity Index[b]	Mortality[c]
C. barbata	S	21·00 ± 2·33	2·36 ± 0·50	38·23 ± 7·46	16·10 ± 5·82	35%
	P	17·20 ± 2·01	3·50 ± 0·52	26·00 ± 3·62	7·77 ± 1·64	
E. aegyptiaca	S	24·82 ± 3·10	2·40 ± 0·50	40·96 ± 5·87	17·78 ± 4·41	23%
	P	18·43 ± 2·43	3·67 ± 0·65	29·50 ± 4·89	8·39 ± 2·47	
E. indica	S	18·80 ± 1·71	3·08 ± 0·62	27·24 ± 5·75	9·44 ± 2·86	58%
	P	15·60 ± 2·63	4·25 ± 0·62	14·92 ± 3·09	3·59 ± 1·02	

Mean ± Standard deviation; S, Sexual reproduction; P, parthenogenetic reproduction.
[a]Number of eggs laid during the life span; [b]Fecundity/preoviposition period; [c]Total percentage of mortality from egg to adult.

pupae. Already mated female pupae mate again with several males that visit the same spikelet. On the contrary, only fully matured adults of *C. indicus* mate for 20–50 s and their males are also polygamic. When pairing, the male grasps the pterothorax of the female with its forelegs, twists its abdomen around 180° beneath the female and moves its copulatory organ till it encounters the genitalia of the female.

In *C. mexicanus*, fecundity is comparatively higher and the reproducing days are more in sexually reproducing individuals. In the laboratory, parthenogenetically and sexually reproducing females lay everyday 2–3 and 3–4 eggs respectively. While the total number of eggs laid by single sexually reproducing female is $38{\cdot}23 \pm 7{\cdot}46$ on *C. barbata*, it is $40{\cdot}96 \pm 5{\cdot}86$ on *E. aegyptiaca* and $27{\cdot}24 \pm 5{\cdot}75$ on *E. indica*, the fecundity index (total number of eggs laid/preoviposition period) being $16{\cdot}1 \pm 5{\cdot}82$, $17{\cdot}78 \pm 4{\cdot}41$ and $9{\cdot}44 \pm 2{\cdot}86$ respectively (table 1). *C. indicus* females oviposit among the floral parts of *A. aspera*, either within the corolla or calyx. Under laboratory conditions these females lay an average of 2–5 eggs per day, with a fecundity of $56 \pm 3{\cdot}05$. The oval eggs of *C. indicus*, with hexagonal chorionic reticulations, measure $144{\cdot}5 \pm 11{\cdot}52$ μ wide and $384{\cdot}12 \pm 23{\cdot}59$ μ long.

3.3 *Life cycle, sex ratio and mortality rates*

Studies on the post embryonic development of *C. mexicanus* were made during February, May, August and November on 3 different host plants, *C. barbata*, *E. aegyptiaca* and *E. indica*. While the time required for the completion of post-embryonic development is shorter in May in all the 3 host plants, the high fecundity and short life cycle in all the seasons is observed in these thrips on *C. barbata* than in the other host plants. Unlike *C. mexicanus*, *C. indicus* does not show seasonal variation in the life cycle and the total duration ranges from 14–19 days, the duration of incubation for I larva, II larva, I pupa and II pupa being 2·57, 3·57, 4·71, 1·00, 2·28 and 1·57 days respectively. Soon after hatching the I instar larvae move from flower to flower to feed on the ovarian tissues of the spikelets of *A. aspera*. After completion of the larval period they conceal themselves within the closed flowers and pupate (figure 1). The duration of each instar of *C. mexicanus* is provided in table 2.

While the sex ratio (female: male) in *C. mexicanus* is 1:1 when inhabiting *C. barbata* and *E. aegyptiaca*, it is 3:2 in *E. indica*. More males are produced during the months of May and August, the sex ratio being 1:4 in all the 3 host plants and fewer males are produced in November changing the sex ratio to 1:2. In the months of April and May, a higher population of males is evident in the field followed by a subsequent decline. Population analysis of *C. indicus* reveals that the females are often predominant and outnumber the males. The sex ratio which remained as 2:1 (female: male) throughout the study period does not get affected by any seasonal variation or biotic influence.

Individual cultures of *C. mexicanus* on *C. barbata*, *E. aegyptiaca* and *E. indica* indicate a higher mortality on *E. indica* (58%) than on *C. barbata* (35%) and *E. aegyptiaca* (23%). In *C. indicus* which infest only *A. aspera* mortality rate is 28% (table 1).

3.4 *Population trends of C. mexicanus and C. indicus*

Under natural conditions though *C. mexicanus* infests all the 3 host plants, *E. aegyptiaca* alone harbours a large population throughout the period of occurrence. The popu-

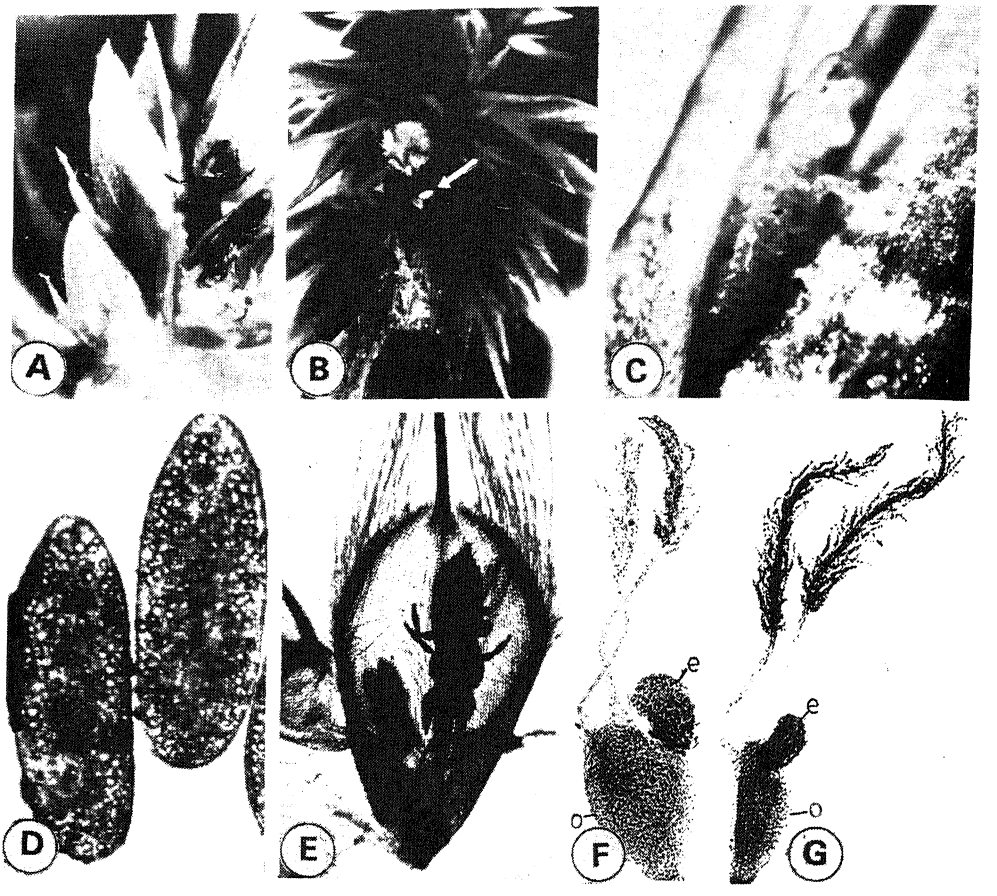

Figure 1. **A.** Adults of *C. indicus* on the inflorescence of *A. aspera*. **B.** Eggs of *C. indicus* inside the flowers of *A. aspera*. **C.** Pupa of *C. indicus* inside the flower of *A. aspera*. **D.** Enlarged view of the eggs of *C. indicus*. **E.** An adult of *C. mexicanus* feeding on the ovary of *C. barbata*. **F.** Egg of *C. mexicanus* partially inserted into the ovary of *E. aegyptiaca*. **G.** Egg of *C. mexicanus* partially inserted into the ovary of *C. barbata*. e, Egg; o, Ovary.

lation generally increases with an increase in temperature and decrease in humidity and in the absence of rainfall. While the population peak of *C. mexicanus* on *C. barbata* is completed by March, on *E. indica* and *E. aegyptiaca* infestation commences only by August and continues till May, the population of males increasing more than 5 folds during March and April on all the 3 hosts. High temperature and low humidity during May initiates the decline of the population. Since the flowers of *E. aegyptiaca* and *E. indica* dry and wither by the end of May, the population of the thrips on these host plants declines. Even though the flowers of *C. barbata* are available throughout the year, the population of *C. mexicanus* disappears by December, but reappears on *E. aegyptiaca* and *E. indica* at a very low level. Rainfall profoundly affects the population of *C. mexicanus* on all the host plants (figure 2).

C. indicus is highly seasonal occurring for about 8 months (October–April). Their incidence on the inflorescence of *A. aspera* is noticed by October and is maintained

Table 2. Life-cycle of *C. mexicanus* on different host plants during different months.

Host plant	Month	Incubation*	I instar*	II instar*	Prepupa*	Pupa*	Total*
C. barbata	Feb.	5·8 ± 0·79	3·5 ± 0·54	7·4 ± 0·51	1·12 ± 0·09	3·5 ± 0·54	21·39 ± 4·11
	May	4·4 ± 0·51	2·5 ± 0·54	5·5 ± 0·54	0·88 ± 0·13	2·7 ± 0·48	15·98 ± 0·95
	Aug.	5·3 ± 0·41	3·4 ± 0·51	6·3 ± 0·48	0·96 ± 0·03	2·6 ± 0·51	18·46 ± 1·17
	Nov.	7·5 ± 0·64	5·1 ± 0·89	7·4 ± 0·51	1·65 ± 0·03	3·8 ± 0·79	25·45 ± 2·11
E. indica	Feb.	7·4 ± 0·51	4·5 ± 0·54	7·8 ± 0·79	1·00 ± 0·00	3·9 ± 0·73	24·60 ± 1·55
	May	5·1 ± 0·25	3·8 ± 0·13	6·7 ± 0·19	0·91 ± 0·00	3·7 ± 0·16	20·04 ± 0·57
	Aug.	5·8 ± 0·25	4·0 ± 0·25	7·4 ± 0·16	0·98 ± 0·01	4·6 ± 0·22	22·78 ± 0·60
	Nov.	7·4 ± 0·16	5·6 ± 0·16	8·3 ± 0·16	1·69 ± 0·03	5·4 ± 0·22	28·49 ± 0·44
E. aegyptiaca	Feb.	6·5 ± 0·51	4·2 ± 0·25	7·8 ± 0·79	1·00 ± 0·00	4·4 ± 0·51	23·90 ± 1·67
	May	5·4 ± 0·51	3·5 ± 0·54	6·1 ± 0·89	0·97 ± 0·03	3·5 ± 0·51	19·46 ± 1·86
	Aug.	5·7 ± 0·66	4·6 ± 0·79	7·1 ± 0·73	1·00 ± 0·00	4·7 ± 0·79	23·10 ± 2·56
	Nov.	7·9 ± 0·74	5·5 ± 0·54	7·7 ± 0·82	1·63 ± 0·09	4·7 ± 0·57	27·36 ± 1·11

Mean ± Standard Deviation *Duration in days.

at a low level till January. In the following months, the population gradually increases and attains peak by April, but declines and disappears by May. Increase in temperature and decrease in the humidity enhances the population build-up of *C. indicus* but a high temperature with low humidity appears to have an adverse effect on the population, showing the preference of *C. indicus* for the warmer months (January–April) to build-up their population. Rainfall adversely affects their population. The availability of the host plant is also an important factor for the population fluctuations of *C. indicus*, the survival of the former to a great extent is affected by the rainfall (figure 3).

3.5 *Damage to seeds of E. aegyptiaca and A. aspera*

Based on the observations on the duration of lifecycle, mortality, average life span and population intensity, *E. aegyptiaca* appears to serve as the primary host for *C. mexicanus* that not only feeds but also inserts its egg into the ovarian tissues. An attempt has therefore been made to study the extent of seed damage in *E. aegyptiaca* by feeding and oviposition of *C. mexicanus*. Adults feed on the milky contents of the 3–6 days young ovaries preventing seed formation. Eggs are laid in the ovarian tissues of 9–14 days old inflorescence, the developing larvae feeding on the ovarian tissues of the spikelet from which they emerged. As many as 9 ovaries/day are consumed by an individual adult with an average consumption of 223·2 ovaries throughout its life span. In the laboratory, individual females lay 2–3 eggs per day producing an average of 38·23 ± 7·46 eggs during their life time. As a result, during its life span, a female damages as many as 261·43 ± 7·46 ovaries of *E. aegyptiaca* both by feeding and oviposition. The adults cause more damage by feeding than the larvae. The overall damage is 70–80% in the laboratory and 20–30% in the field. The assessment of the damage in the field is based on the number of ovaries of the spikelet of *E. aegyptiaca* containing eggs and immature stages of thrips. A significant positive correlation ($r = 0·87$) is obtained between the total number of ovaries in an inflorescence and the number of ovaries damaged by *C. mexicanus* (figure 4).

Both adults and larvae of *C. indicus* feed on the outer surface of the ovarian tissues

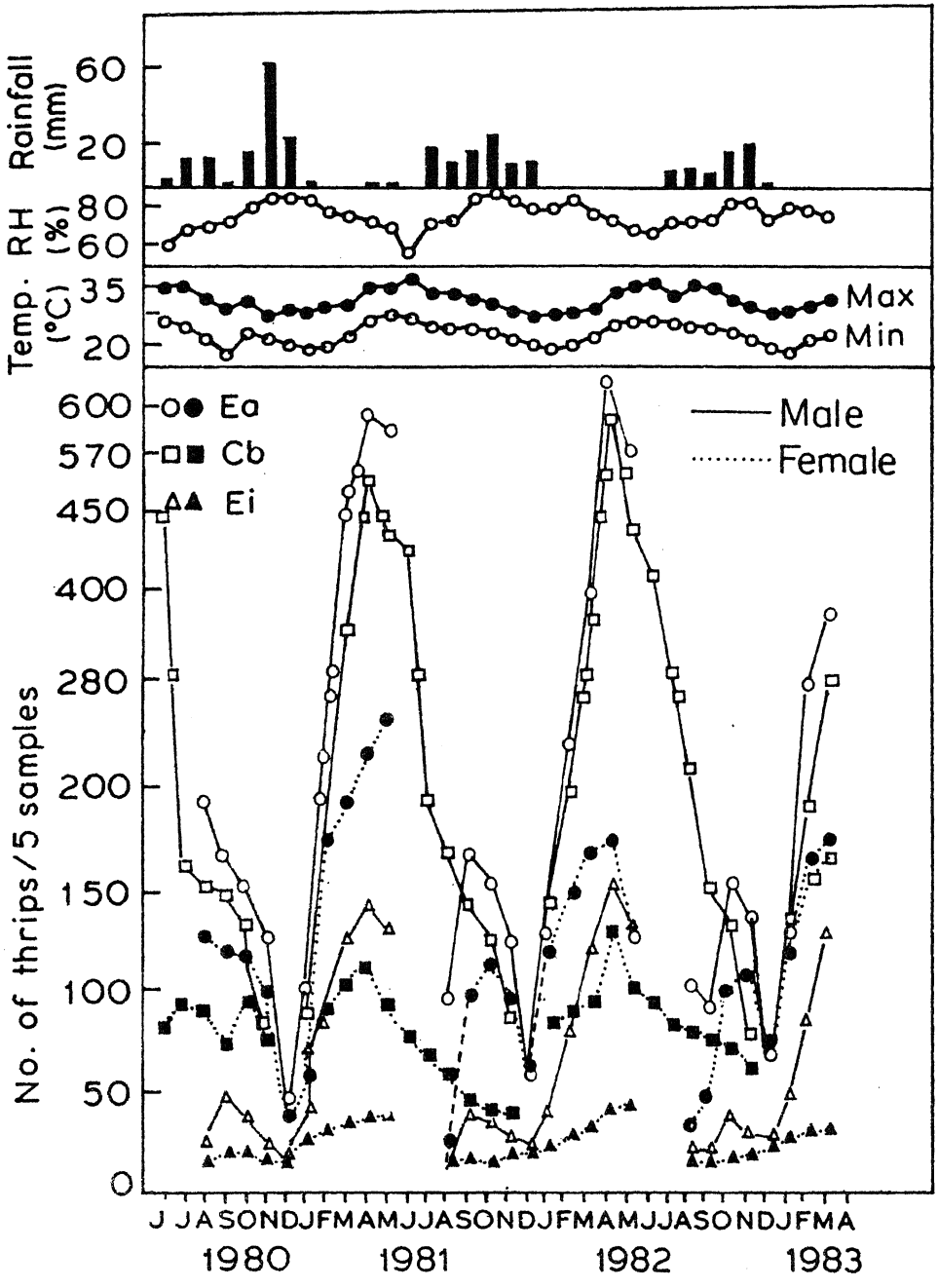

Figure 2. Population dynamics of *C. mexicanus* on 3 different host plants of *E. aegyptiaca* (Ea), *C. barbata* (Cb) and *E. indica* (Ei).

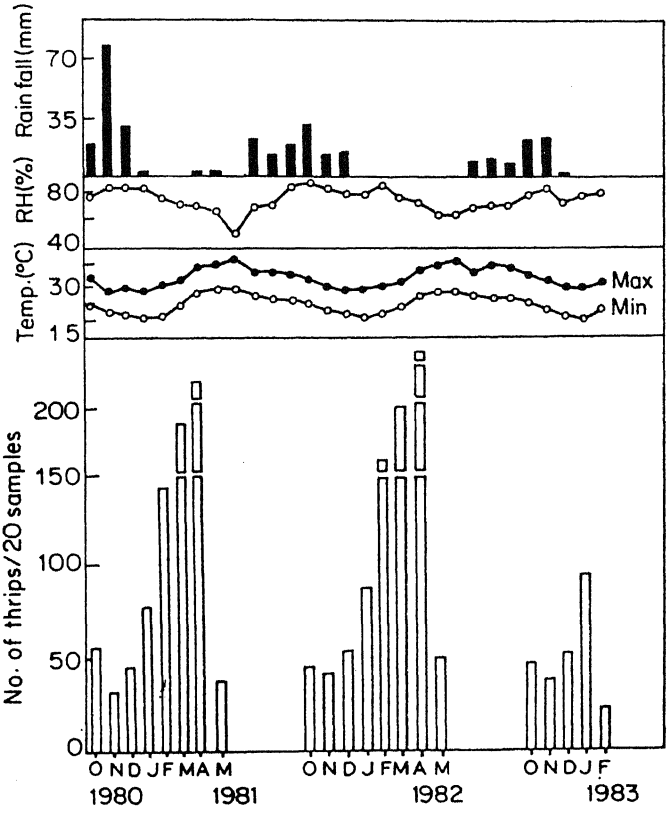

Figure 3. Population dynamics of *C. indicus* on *A. aspera.*

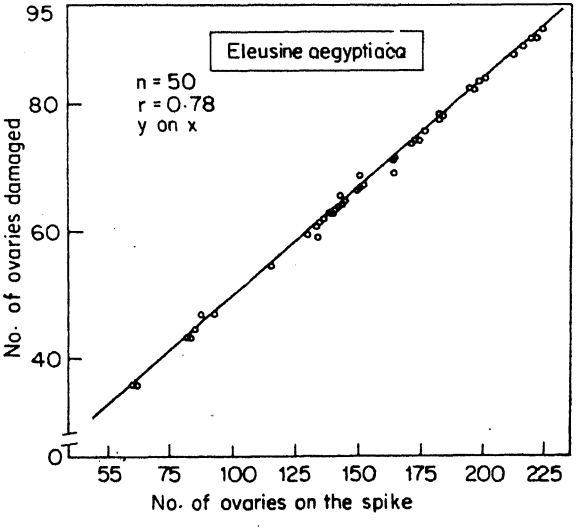

Figure 4. Ovarian damage due to *C. mexicanus* infestation.

of *A. aspera* preventing subsequent seed production. As many as 5 individuals are found inside single spikelet and before they get enclosed by periants the first larvae move to fresh non-infested flowers to continue their feeding activity, affecting more and more ovaries of the spikelets. The randomly feeding adults damage 46–53% of ovaries of *A. aspera*. Statistical analysis reveals a significant positive correlation ($r = 0{\cdot}82$) between the total number of ovaries in spike and the number of ovaries damaged by *C. indicus* (figure 5).

4. Discussion

Sexual and parthenogenetic reproduction appear to be typical of *C. mexicanus*, and arrhenotoxy is the principal mode of parthenogenetic reproduction, resulting in a dense population of males. More males are produced during summer when the temperature is high. Loan and Holdaway (1955) have also reported initiation of parthenogenesis in *Haplothrips niger* (Osborne) only at temperatures above 25°C and not below. These observations reveal that some thrips species resort to parthenogenetic reproduction at higher temperatures. In *C. indicus* however, the females always outnumber the males due to the absence of parthenogenesis and do not exhibit any such variation in sex ratio with respect to seasonal changes. As observed in the case of *Limothrips denticornis* Haliday (Pussard-Radulesco 1930) adult males of *C. mexicanus* also mate with premature females. But unlike in *L. denticornis*, *C. mexicanus* neither exhibit hibernation nor store the sperms in prepupal spermatheca, but exhaust all the sperms once they attain maturity. As has been reported in many terebrantians (Riherd 1954; Doull 1956; Ananthakrishnan and Thirumalai 1978), *C. mexicanus* oviposit their eggs inside the ovarian tissues of the host plants. Due to lack of ovipositor, *C. indicus* always lays its eggs only on the host surfaces. It is well known that, the rate of development of insects does not increase proportionately with the rise in temperature throughout the range suitable

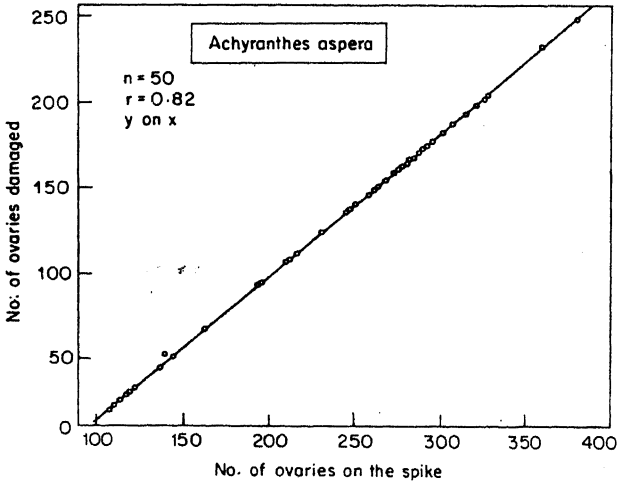

Figure 5. Ovarian damage due to *C. indicus* infestation.

for development, but is faster at the optimum temperature, any deviation slowing down the rate of growth (Lewis 1973). It is also well recognised that in thrips not only seasonal temperature changes but also the constant and fluctuation temperatures affect the development of the species. In *Anaphothrips obscurus* (Muller), the duration of life cycle varies from 12 days in summer to 30 days in spring. *Thrips tabaci* Lindeman takes 11·2 days to complete its life cycle (from egg to adult) at a constant temperature of 30°C whereas under fluctuating temperature with a mean of 30·8°C, it takes 13·9 days (Lall and Singh 1968). In *C. mexicanus*, which reproduces throughout the year, summer with high temperatures appears to be a favourable season for development.

Increasing temperatures and decreasing relative humidity enhance the population build-up in *C. indicus*. Similar observations have also been made in *C. mexicanus* (Ananthakrishnan and Thirumalai 1978). As the flowers of *E. aegyptiaca* and *E. indica* dry up by the end of May, no further populations of *C. mexicanus* are observable. Inspite of the occurrence of flowers of *C. barbata* throughout the year, the prevalence of low temperature and high humidity during rainy season cause the insects to disappear on this host. This rainfall appears to have a profound and adverse effect on *C. mexicanus* and *C. indicus* populations.

Host plants have a significant influence on the biology of *C. mexicanus*, a seed infesting species, whereas this is not so in the monophagous *C. indicus*. Though Ananthakrishnan and Thirumalai (1977) have recorded *C. barbata* as the primary host for *C. mexicanus*, comparative studies on the population and reproductive biology of this thrips on the 3 different host plants show *E. aegyptiaca* to be the most suitable and preferred host of *C. mexicanus*, on which it exhibits high fecundity, long life span and low mortality rate (table 1). *C. barbata* and *E. indica* may be considered as secondary and tertiary hosts respectively.

Different species of *Chirothrips* are known to feed on the ovarian tissues of many grass species destroying a large number of seeds (Lewis 1973). The feeding of *C. indicus* larvae on the ovarian tissues restricted to the oviposited sites and that of the adults on these tissues at very many places results in extensive loss of seed production to about 70–80% in *E. aegyptiaca*. Similarly *C. mexicanus* is reported to cause 60% and 5–10% on *C. barbata* and on *Pennisetum typhoides*, respectively (Ananthakrishnan and Thirumalai 1977). *C. hamatus* and *C. pallidicornis* species closely related to *C. mexicanus*, have also been reported to cause 25 and 30% seed loss in meadow foxtail grass and cocksfoot grass respectively (Hukkinen 1936; Doull 1956). Statistically there is a significant and direct correlation between the number of ovaries in an inflorescence and the number of ovaries damaged by *C. mexicanus* on *E. aegyptiaca*.

The fecundity of sexually and parthenogenetically reproducing individuals of *C. mexicanus* is always higher when they feed and develop on *E. aegyptiaca* than on *C. barbata* and *E. indica*. These thrips also complete their development faster, with low mortality rate, always maintaining a high populations on *E. aegyptiaca* than the other hosts. Hence *E. aegyptiaca* appears to be a more suitable and preferred host. Based on the assessment of these parameters it can also be said that *E. indica* is a less preferred host than *C. barbata*. But these hosts enable the thrips to maintain their population at a very low level throughout their infestation.

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Light and electron microscopic observations on lipid droplets of the sebaceous flank gland of the shrew, *Suncus murinus viridescens* (Blyth)

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Abstract. Light and electron microscopic investigations have been made on the lipid droplets of the sebaceous flank gland alveoli of the Indian musk shrew, *Suncus murinus viridescens*. The undifferentiated sebaceous cells can be distinguished from the differentiating and differentiated cells by the absence of lipid droplets in the former. Adjacent droplets in the differentiating cells fuse to form larger droplets. Lipid droplets are also synthesized in the differentiating cells. Large accumulation of lipid droplets are seen in well differentiated cells. The cells of the inner layer of the sebaceous alveoli disintegrate and the holocrine products are liberated into the lumen. The probable role of mitochondria and polyribosomes in the synthesis and development of lipid droplets in the sebaceous cells of the flank gland is discussed.

Keywords. Indian musk shrew; *Suncus murinus viridescens*; flank gland; sebaceous alveoli; lipid droplets; light and electron microscopic investigations.

1. Introduction

Although extensive investigations on specialized integumentary glands of mammals have been carried out during the past few years (for review, Müller-Schwarze 1983), skin glands of only a few of the Indian mammals have been studied so far (for review, Balakrishnan and Alexander 1985). Balakrishnan *et al* (1986) have studied the structure of the specialized cutaneous glands of the Indian musk shrew, *Suncus murinus viridescens* in detail. Sebaceous glandular alveoli form the bulk of the flank gland of this species with a few sudoriferous tubules located mainly at the glandular periphery. Each alveoli has a central lumen where the glandular secretory materials are temporarily stored and later discharged to the exterior, especially during the process of scent marking (Balakrishnan and Alexander 1985).

Lipids form the major component of this glandular secretion of the musk shrew (Balakrishnan and Alexander 1985). These substances have specific behavioural relevance during social interactions. The present report deals with light and electron microscopic observations on the formation and development of the lipid secretory droplets in the sebaceous alveoli of the flank gland of *S. m. viridescens*.

2. Material and methods

Adult shrews (9 males and 8 females) were used for the present investigation. They were trapped live on the University Campus, Kariavattom, and were kept in individual wire-mesh cages for 2–3 days on a regular supply of minced beef sprinkled with shark liver oil and tap water, *ad libitum*. Fur covering the flank glands was shaved off using a fine blade and the glands were dissected out for processing, for histological, histochemical and ultrastructural studies. Glands from one side from 5

males and 5 females were fixed in Bouin's fluid, processed, paraffin sections were cut at 5 μm and stained in haematoxylin and eosin. The glands from the other side were fixed in 10% formalin, gelatin blocks were prepared and the sections were cut at 8–10 μm on a Model CTD International Harris Cryostat at $-20°C$. These sections were stained with Sudan black-B.

For electron microscopic investigations, the glands from 4 males and 3 females were dissected out under ether anaesthesia. Thin slices of the glandular tissues were fixed in 2·5% glutaraldehyde in 0·1 M phosphate buffer (pH 7·2–7·3). The tissues were washed in the buffer for 2 h and post fixed in 1% osmium tetroxide in 0·1 M phosphate buffer. The samples were then carried to the USSR in the same buffer and dehydrated using alcohol and acetone in the ascending grades and embedded in Epon 812. Ultrathin and 1 μm sections were cut using glass knives in LKB Ultrotomes III and IV. Ultrathin sections were mounted on formvar-coated grids and stained with uranyl acetate and lead citrate (Reynolds 1963) and examined under a JOEL JEM 100 C electron microscope at an accelerating voltage of 80 kV. One microne section was prepared and stained with toluidine blue and observed under a light microscope.

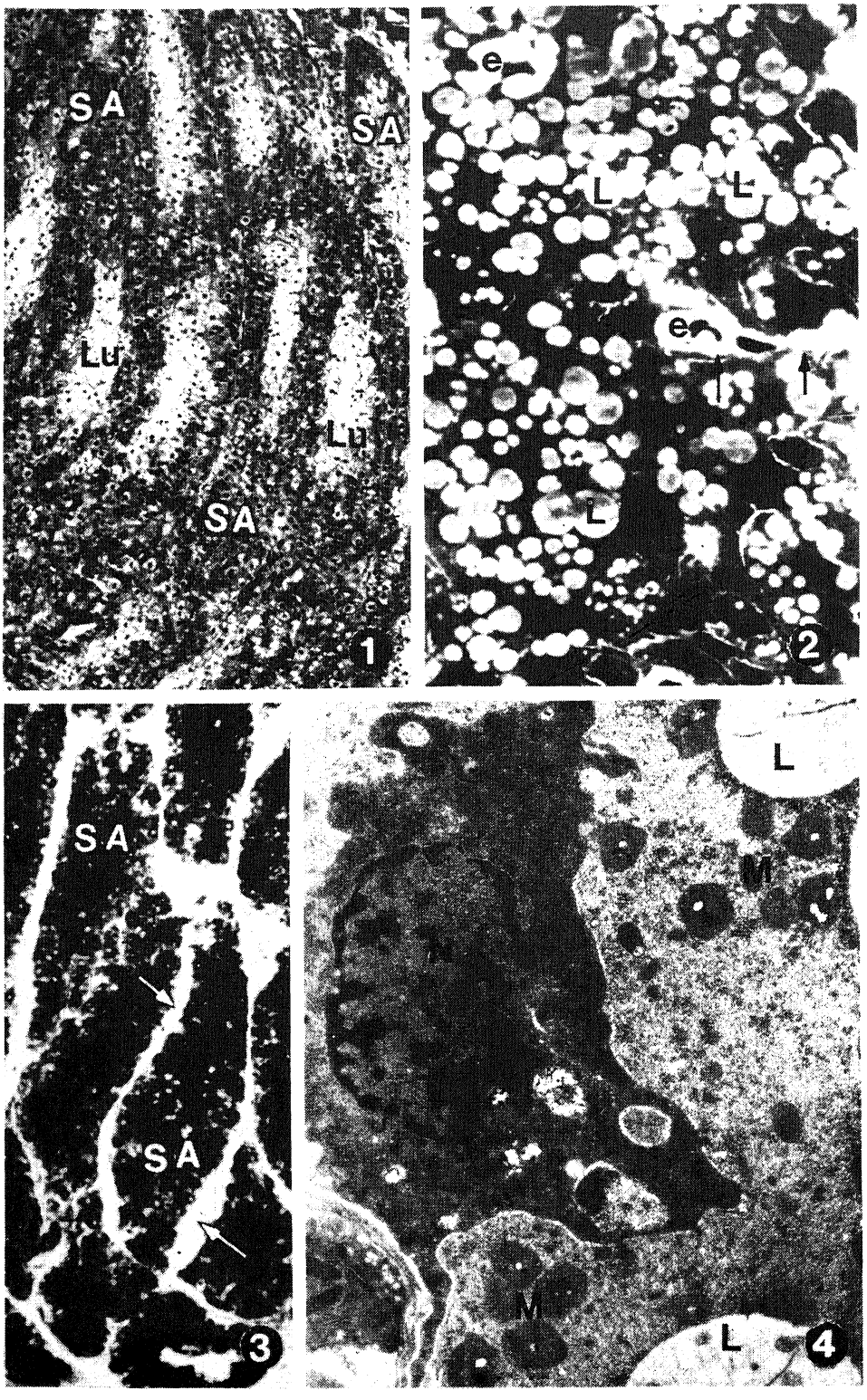
3. Results

Each of the sebaceous alveoli of the flank gland consists of about 4–6 layers of cells (figure 1). The peripheral layer of the alveoli consists of undifferentiated cells. The middle layers consist of differentiating cells and the inner layers consist of well differentiated and disintegrating cells.

Light microscopic observations revealed that the cells in the outer-most layer of the sebaceous alveoli are devoid of lipid droplets. Lipid droplets are seen in large numbers in the cells of the middle and inner layers of the alveoli (figure 2). The cells of the inner-most layer can be well distinguished by their holocrine secretory activity; these cells disintegrate *in situ* and discharge their contents into the lumen of the sebaceous alveoli in the form of large number of well developed droplets. During development and differentiation the lipid droplets enlarge with their opaque contents. Cells in the inner layers of the alveoli contain large number of such droplets. The glandular secretion is eliminated to the exterior through the special ducts of the alveoli.

Histochemical studies have revealed that the secretory products of the sebaceous cells of the flank gland are predominantly lipoid in nature. The secretory products in general stained deeply with Sudan black-B. Lipids were not detected in appreciable

Figures 1–4. **1.** Light micrograph of the sebaceous flank gland alveoli of a male shrew. Note the arrangement of cells in the alveoli. 5 μm haematoxylin × eosin (× 100). **2.** One microne section of the flank gland of a male shrew showing the pattern of distribution of lipid droplets in the sebaceous alveoli. Note the greater accumulation of lipid droplets in the inner cells and in the lumen of sebaceous alveoli. Capillaries ending in the epithelial wall (indicated by arrows) are seen with erythrocytes (× 1000). **3.** Flank gland alveoli of a male shrew showing the absence of lipids in the connective tissue envelope of the alveoli (arrows). The peripheral cells contain less amount of lipids as compared to the inner cells. Sudan black-B (× 400). **4.** Part of a sebaceous alveoli of the flank gland of a female shrew showing the undifferentiated cell on the left half with dense cytoplasm when compared to that of the differentiated cell on the right (× 22,000).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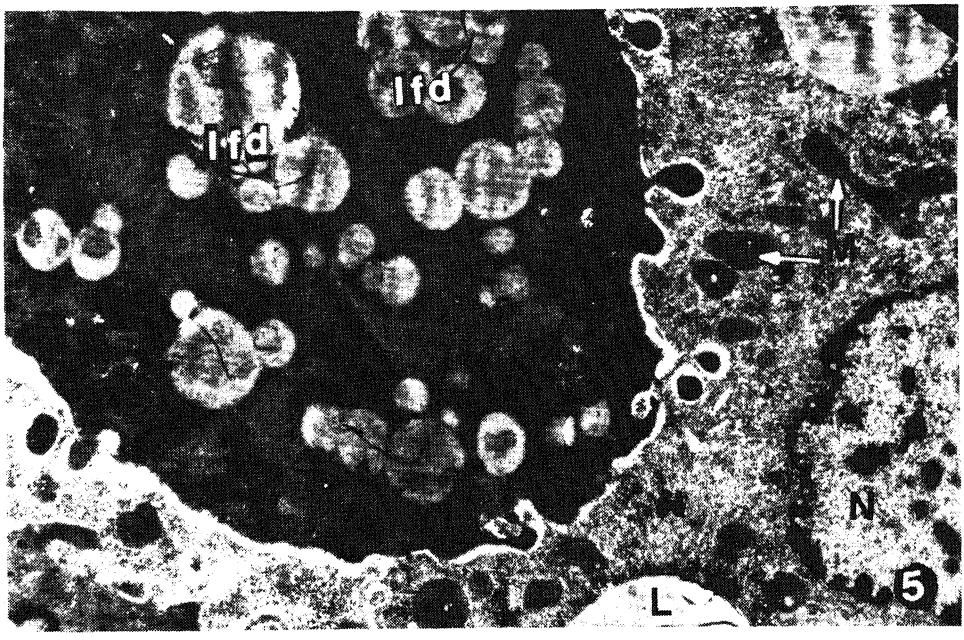
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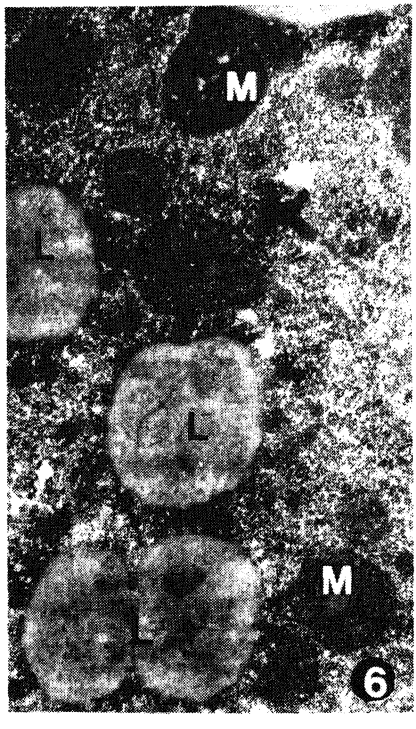
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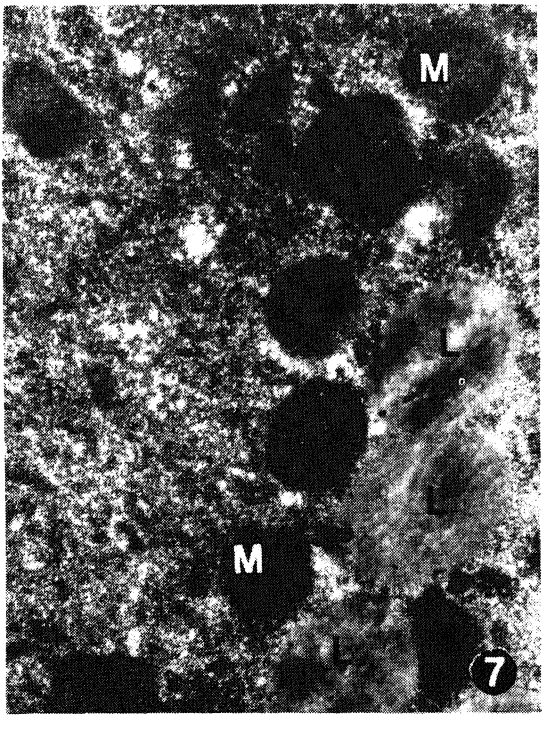
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quantity in the outer covering and in the peripheral layer of the alveoli. Heavy lipid accumulation was noted in the lumen and in the cells of the sebaceous alveoli (figure 3).

No difference in the histochemical and ultrastructural features of the sebaceous cells in the flank gland between males and females could be detected.

The undifferentiated cells of the sebaceous alveoli can also be distinguished from the differentiated cells by the difference in the intensity of the cytoplasm. The cytoplasm of undifferentiated cells are denser as compared to that of the differentiated cells (figure 4). The differentiating cells have lipid droplets in various stages of development. Adjacent smaller droplets fuse to form larger droplets during cellular differentiation and maturation (figures 5 and 6).

Large number of mitochondria are observed around the lipid droplets both in the differentiating and in the well differentiated sebaceous cells of the flank gland (figures 6–8). Most of the mitochondria in the differentiating cells are large, round and swollen (figure 7), whereas those of the differentiated and disintegrating cells are smaller and oval or elongated (figures 8 and 9). Polyribosomes are also seen accumulated around lipid droplets (figures 6–9). In the well developed cells, lipid droplets occupy most of the area in the cytoplasm (figure 9).

4. Discussion

The sebaceous alveoli of the flank gland of *S. m. viridescens* show cells in different stages of development, which can be identified on the basis of presence and larger accumulation of lipid droplets. New layers of secretory cells grow from the periphery of the alveoli, develop, differentiate and subsequently disintegrate and discharge their contents into the lumen by holocrine secretion (Kurosumi 1961).

In the course of lipogenesis in the mouse Meibomian gland, Gorgas and Völkl (1984) have demonstrated 4 phases, (i) formation of large number of small, spherical lipoid droplets, (ii) enlargement of these droplets, (iii) further expansion in the volume and accumulation of an electron opaque material in it and (iv) the lethal phase. It is also reported that the composition of cellular lipids changes during the development and differentiation of sebaceous cells (Potter *et al* 1979; Wheatley *et al* 1979). In the present investigation, it was noticed that during cellular differentiation, smaller lipid droplets fused together to form larger droplets (figures 5 and 6). However, in differentiating and well differentiated sebaceous cells, both small and large lipid droplets were noticed. This may be due to the fact that new lipid droplets

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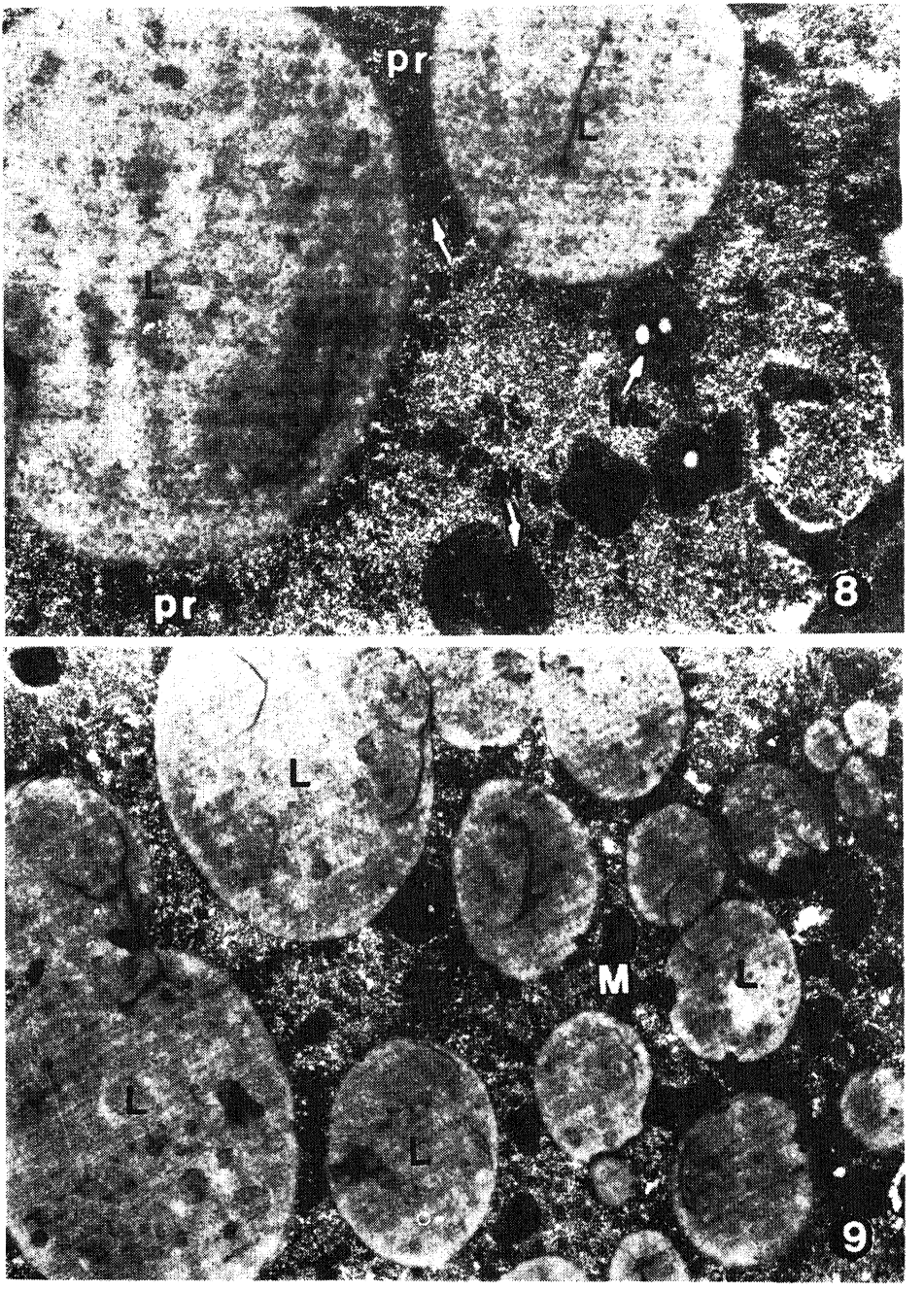
Figures 5–7. 5. Part of a sebaceous alveoli of the flank gland of a female shrew showing a differentiating and a differentiated cells. The adjacent smaller lipid droplets synthesized during the process of differentiation are fused to form larger droplets of the differentiated cells (× 22,000). **6.** Part of a differentiating cell of the sebaceous alveoli of a female shrew showing the process of fusion of lipid droplets. Large, round and swollen mitochondria are seen near the developing lipid droplets. Polyribosomes are accumulated on the periphery of the lipid droplets (× 22,000). **7.** Part of a differentiating cell of the sebaceous alveoli of the flank gland of a female shrew showing the close association of large, round and swollen mitochondria with developing lipid droplets (× 22,000).



Figures 8–9. 8. Part of the well differentiated cell of the sebaceous alveoli of a female shrew flank gland showing large number of polyribosomes attached to the periphery of the lipid droplets. The mitochondria in the developed cells are comparatively smaller, and of oval, elongated or of irregular shapes (× 22,000). **9.** Part of a disintegrating cell of the sebaceous alveoli of the flank gland of a female shrew. Lipid droplets occupy most of the area in the cytoplasm of the developed and disintegrating cells. The mitochondria are small and oval, elongated or irregular in shape (× 22,000).

are continuously synthesized by these cells as observed by Wooding (1980) in the sebaceous cells of the Hardarian glands of rabbit.

The close association of lipid droplets with mitochondria having the characteristic electron dense inclusions in the sebaceous flank gland cells of the shrew has already been reported (Balakrishnan *et al* 1986). It is also suggested that the mitochondria are engaged in lipid metabolism in specialized glandular cells (Bjersing 1967; Fawcett 1981; Jenkinson *et al* 1985). Balakrishnan *et al* (1986) have also observed that polyribosomes are situated around the periphery of the lipid droplets which may contribute necessary enzymes for the synthesis and development of lipid droplets.

Well developed capillaries in the peripheral cells of the sebaceous alveoli of the shrew flank gland (figure 2) facilitate the flow of raw materials from the surrounding media, and the finger-like protrusions of the cellular periphery at the level of capillaries (Balakrishnan *et al* 1986) provide additional surface for absorption and ingestion of more raw materials into these secretory cells.

Since steroid hormones have profound influence on the structure and function of the sebaceous glands in a number of species of mammals (Bell 1974; Strauss *et al* 1976), ultrastructural investigations on the sebaceous flank gland tissues of castrated and hormone administered shrews are likely to yield valuable information on the lipogenesis in the sebaceous cells.

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Abbreviations used in the figures: SA, Sebaceous alveoli; Lu, lumen of the sebaceous alveoli; L, lipid droplets; e, erythrocyte; N, nucleus; M, mitochondria; lfd, lipid droplets in the process of fusion and development; pr, polyribosomes.

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Quantitative analysis of carbohydrases in the crystalline style of some intertidal bivalve molluscs

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Abstract. Amylase activity in different pH ranging from 3·6 to 8·0 and activity of sucrose, maltose, trehalase, raffinase, lactase, glycogenase, pectinase, chitinase, carboxy methyl cellulase and cellulase at their respective optimum pH were quantitatively estimated in the crystalline style of intertidal bivalves; *Crassostrea madrasensis*, *Meretrix meretrix*, *Meretrix casta*, *Katelysia opima* and *Donax cuneatus*. Amylase was the most active among all enzymes tested. A maximum activity of 137·5 μg/mg/h was recorded in the style of *Katelysia opima* while the activity was minimal in *Donax cuneatus* (39·3 μg/mg/h). Two pH optima were recorded for style amylase of *Crassostrea madrasensis* (6·0 and 6·8) and *Katelysia opima* (5·6 and 6·8). But the activity was higher at higher optimum pH. The enzymes hydrolysing oligosaccharides were weak in most cases or absent. The enzymes acting on reserve polysaccharides such as amylase and glycogenase showed maximum activity in the species tested. However the activity of enzymes degrading structural polysaccharides such as cellulase, carboxy methyl cellulase, pectinase and chitinase was either at a low level or absent. The role of the style enzymes in the extracellular digestion of bivalves is discussed.

Keywords. Bivalve molluscs; crystalline style; carbohydrases; *crassostrea*; *meretrix*; *katelysia*; *donax*.

1. Introduction

Consequent to the loss of salivary gland in the evolution of Bivalvia, crystalline style might have developed as a source of supply of starch splitting enzyme (Yonge 1923). Two groups of digestive enzymes, carbohydrases and lipases have been identified in the crystalline style of bivalves (Owen 1966, 1974; Morton 1983). The presence of amylase in the style of bivalves was reported by Morton (1983). Other carbohydrases like glucosidases and galactosidases and structural polysaccharases like cellulase, carboxy methyl cellulase and chitinase were also shown to be present in the style of many bivalves and this subject has been reviewed by van Weel (1961), Owen (1966, 1974) and Morton (1983). However, quantitative measurements on the activity of carbohydrases of the style were limited to the work of Horiuchi and Lane (1966), Kristensen (1972), Wojtowics (1972) and Langton and Gobbott (1974). The present work was undertaken to quantify the activity of style carbohydrases and to understand the nature of the process of digestion in some intertidal bivalves.

2. Materials and methods

Crassostrea madrasensis (Preston), *Meretrix meretrix* (Linnaeus), *Meretrix casta* (Chemntiz) and *Katelysia opima* (Gmelin) were collected from the mud flats of Vellar

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estuary joining Porto Novo sea shore (11°29′ N lat. and 79°46′ E long.) and *Donax cuneatus* (Linnaeus) from the marine intertidal zone.

2.1 *Preparation of style extract*

Aqueous extract of the style was prepared by homogenising it with distilled water using an all glass homogeniser. After centrifugation for 15 min at 20,000 g the supernatant was used as enzyme extract. The strength of the extract was 3%.

2.2 *Quantitative estimation of amylase activity in different pH*

A reaction mixture of 1 ml of 1% starch, 1 ml of style extract and 2 ml of buffer was incubated for 2 h at different pH ranging from 3·6–8·0. Two drops of toluene were added to cover the surface of the reaction mixture in test tube to prevent contamination from air. The amount of reducing sugars released by amylase activity was determined by Somogyi-Nelson's photometric method (Nelson 1944). The extinction was measured at 540 nm using distilled water as reference in a spectrophotometer (Spectronic–20). The amount of glucose released was calculated from the calibration curve constructed with standard D-glucose. The protein of the style extract was determined colorimetrically by the method of Raymont *et al* (1964). The enzyme activity was expressed as μg glucose/mg protein/h (μg/mg/h). Control experiments were run simultaneously with heat inactivated style extract. Three estimations were made for each species and the mean values used.

2.3 *Quantitative estimation of activity of other carbohydrases*

The substrates used in these experiments were 2% sucrose, 2% maltose, 1% trehalose, 2% raffinose, 2% lactose, 1% glycogen, 1% pectin, 1% chitin, 1% sodium carboxy methyl cellulose and 1% cellulose. The activity of the different carbohydrases was estimated by the method described above. However, the reaction mixtures were incubated for 8 h and at the optimal pH of the enzyme in question as described by Horiuchi (1963).

2.4 *Buffers*

0·2 M acetate buffer was used to maintain the pH in the range of 3·6–5·6 and 0·2 M sodium phosphate buffer in the range of 6·0–8·0.

3. Results

The amylase activity at different pH and activity of other carbohydrases in their respective optimum pH in the style extract of 5 bivalves species are presented in figures 1 and 2. The activity profile of amylase and other carbohydrases are described.

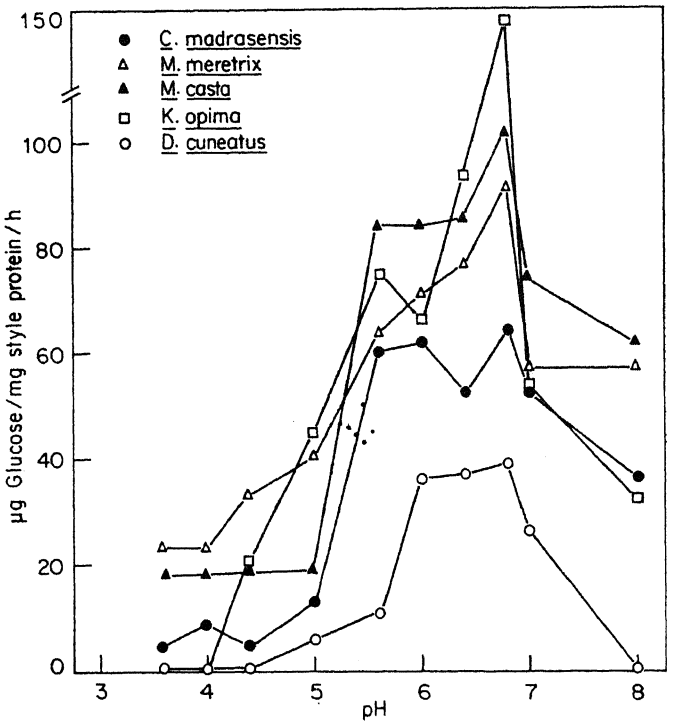

Figure 1. Amylase activity in the crystalline style of the tested bivalve species in relation to pH.

3.1 *C. madrasensis*

Considerable amylase activity was recorded in the style extract of this oyster in the pH range between 5·6 and 7·0 with peak activity at pH 6·0 (62·15 µg/mg/h) and at pH 6·8 (64·5 µg/mg/h). The style extract of this oyster was found to hydrolyse oligosaccharides like sucrose, trehalose, raffinose and polysaccharides like carboxy methyl cellulose and glycogen. The hydrolysis of glycogen was faster (9·6 µg/mg/h) than the hydrolysis of other carbohydrates. Sucrose activity was observed only in *C. madrasensis* and absent in others.

3.2 *M. meretrix*

The optimum amylolytic activity of the style extract of this clam was found at pH 5·6 (64·0 µg/mg/h) and at pH 6·8 (91·0 µg/mg/h). However, the hydrolysis of the starch was spread over the pH range 5·6–8·0. In *M. meretrix* style extract hydrolysed two oligosaccharides, maltose and trehalose and two polysaccharides, glycogen and carboxy methyl cellulose.

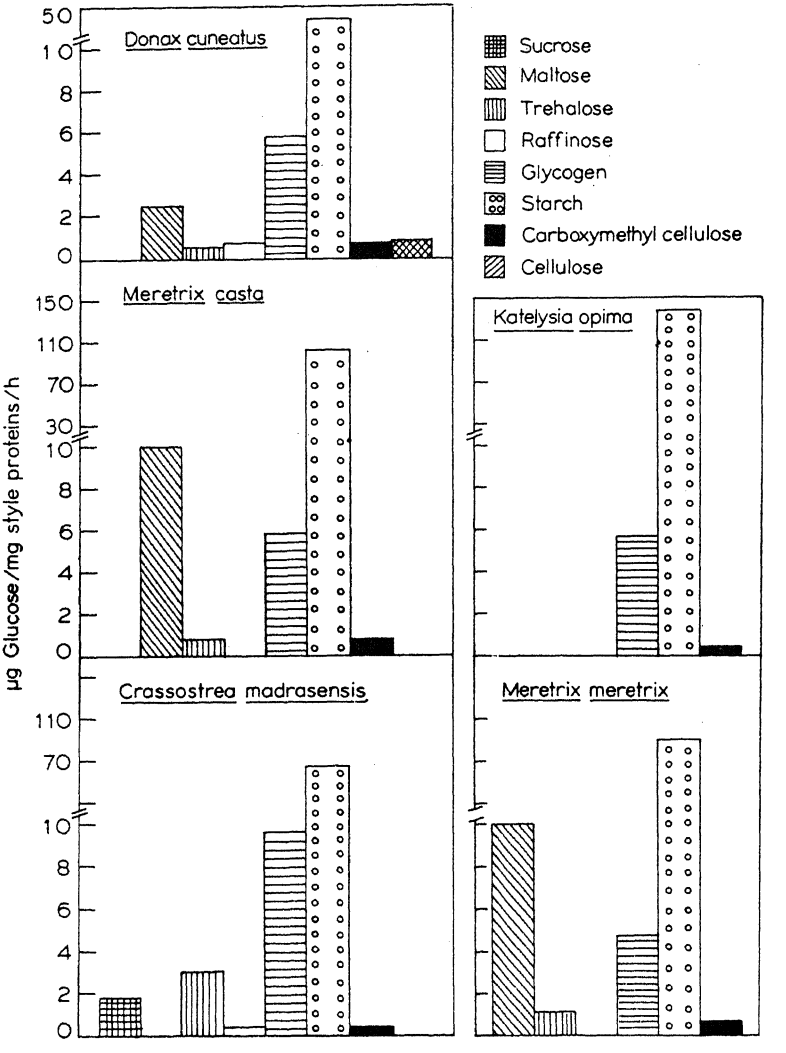

Figure 2. Histograms showing the activity of carbohydrases in the crystalline style of the tested bivalve species.

3.3 *M. casta*

The activity profile for amylase and other carbohydrases of *M. casta* was almost similar to that of *M. meretrix*; however there is a single pH optimum in *M. casta.*

3.4 *K. opima*

There were two pH optima (5·6 and 6·8) in which maximal amylase activity was measured. Of all the bivalves tested the highest rate of amylolysis (137·5 µg/mg/h) was recorded at the optimum pH of 6·8 and at the lower pH optimum of 5·6, the

style extract released 75·1 μg/mg/h. Only two polysaccharides, carboxy methyl cellulose and glycogen were also hydrolysed.

3.5 *D. cuneatus*

Amylase of *D. cuneatus* style was observed to have a lowest level of activity (39·3 μg/mg/h) at the optimum pH of 6·8 when compared with the activity of the style amylase of other species. *Donax* style extract also hydrolysed 3 oligosaccharides, maltose, trehalose and raffinose and 3 polysaccharides, cellulose, carboxy methyl cellulose and glycogen, as in other species the rate of glycogen hydrolysis (5·8 μg/mg/h) was significantly at a higher level.

4. Discussion

Styles of all the tested bivalves maintained a high level of amylase activity but the rate of activity varied among the tested species. A maximum activity of 137·5 μg/mg/h was recorded in the style extract of *K. opima*. The activity was minimal in *D. cuneatus* (39·3 μg/mg/h). In general the result indicated that the activity of the style amylase was always higher in venerids (*M. meretrix*, *M. casta* and *K. opima*) than in ostreid (*C. madrasensis*) or in donacid (*D. cuneatus*). van Weel (1961) and Owen (1966, 1974) also showed variation in amylase activity among some bivalve species. Wojtowics (1972) quantified the amylase activity in the style and digestive diverticula and showed that the activity was higher in the style than in digestive diverticula.

Two pH optima were recorded for the style amylase of *C. madrasensis* (6·0 and 6·8) and *K. opima* (5·6 and 6·8). The occurrence of two pH optima is not uncommon. Hashimoto and Kijima (1956) in *Mactra veneriformis*, Dinamani (1957) in *Villorita cyprinoides*, Black and Pengelley (1964) in *C. virginica* and Horiuchi and Lane (1966) in *Strambus gigas* also reported two pH optima for the style amylase. Black and Pengelley (1964) showed the presence of an amylase with a single pH optimum in the style of oyster larva but the style of the adult contained a second amylase with different pH optimum. In all the species tested with two pH optima for amylase, the style amylase was more active always at higher pH optimum (6·8). Similar observation was also reported by Horiuchi and Lane (1966). Our results indicate that hydrolysis of oligosaccharides in most cases were weak or absent. But the style contained more active enzymes for the hydrolysis of reserve polysaccharides such as starch and glycogen than for structural polysaccharides like cellulose, pectin and chitin. Next to amylase, glycogenase showed a higher rate of hydrolysis in all the species analysed. The glycogenase is one of the earliest known style enzymes and reported to be present in the style of many bivalves (Horiuchi and Lane 1966; Kristensen 1972; Mathers 1973). Mathers (1973) was of the opinion that the style amylase which hydrolysed glycogen was probably the same that also hydrolysed starch, amylase and amylopectin.

The present study also indicates the distribution pattern of cellulolytic enzymes. A true cellulase was recorded only in *D. cuneatus* while the poly-glucosidase capable of digesting carboxy methyl cellulose was recorded in all the species analysed. A similar distribution pattern for cellulolytic enzymes was also reported in literature (Payne *et*

al 1972; Morton 1978). The study of the style carbohydrases of the bivalve species throw some light on the basic nature of digestion in general and extracellular digestion in particular. Since these bivalves largely feed on phytoplankton and organic particulate materials of plant origin (Purchon 1968; Crosby and Reid 1971), they are by and large herbivores. Herbivorous animals generally have more active carbohydrases than carnivores have (Prosser and Brown 1961). As the salivary glands do not exist in filter feeding Bivalvia (Yonge 1923) a compensatory source of carbohydrases is inevitable. Adaptively the development of crystalline style in bivalves provides a rich source of carbohydrases which initiate the extracellular digestion in the stomach. Further, the more active style enzymes are amylase, glycogenase, cellulase and polyglucosidase (present study) and laminarinase (Sova *et al* 1970; Wojtowics 1972) all of which are designed to degrade sugars of high molecular weight. The situation implies that the crystalline style initiates extracellular digestion through the mechanical trituration of food and release of enzymes for the degradation of high molecular weight polysaccharides in the stomach lumen while the final break down and absorption take place in the digestive diverticula.

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Glycogen level and glycogen phosphorylase activity in the eggs of silkworm *Bombyx mori* L.

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Abstract. The glycogen content of the eggs of silkworm *Bombyx mori* L. was analysed at intervals during the course of embryonic development and diapause. Changes in the glycogen levels were also examined in acid treated eggs in which diapause has been broken. These studies showed significant differences in glycogen utilisation pattern in the diapause and non-diapause eggs. The activity of the enzyme glycogen phosphorylase was also found to exhibit different patterns in the two types of eggs. Our studies indicate that this enzyme plays an important role in the metabolic changes which occur during diapause in silkworm eggs.

Keywords. *Bombyx mori*; glycogen; glycogen phosphorylase.

1. Introduction

Glycogen is stored in the eggs of the silkworm *Bombyx mori* L. and is made use of as and when energy is required during the course of diapause as well as embryonic development (Chino 1957). In many other insects also utilisation of glycogen has been found to exhibit a definite pattern which is indicative of metabolic regulation corresponding to the needs of growth and activity (Karlson and Sekeris 1964).

The embryonic development in *B. mori* is interesting because in certain races the embryonic development proceeds uninterrupted while in some, it is characterized by diapause and the diapause eggs can be artificially treated to continue embryonic development without interruption. Therefore, silkworm eggs provide an ideal material for examining the adaptability of glycogen metabolism and its role in diapause. There have been some studies earlier, demonstrating the changes in the glycogen content in silkworm eggs during diapause (Chino 1957, 1958). During the present studies, changes in the glycogen levels during the embryonic development in locally available non-diapause eggs have been examined in detail. Changes in glycogen level in diapause eggs during the course of diapause and also following acid treatment to break diapause have been studied. These studies would explain whether differences, if any, in the pattern of glycogen utilisation in diapause and non-diapause eggs are due to the phenomenon of diapause or due to any inherent differences in these two types of eggs.

The initial and key control step in the utilisation of glycogen is a phosphorolytic reaction catalysed by the enzyme glycogen phosphorylase. Glycogen phosphorylase is an allosteric enzyme capable of occurring in two forms namely an active a-phosphorylase and an inactive b-phosphorylase which are interconvertible (Steele 1982). The vertebrate phosphorylases have been extensively studied (Graves and Wang 1972; Busby and Radda 1976). Similar studies have been carried out in a number of insects and these studies have been reviewed by Steele (1982). However,

not much is known about this enzyme in silkworms. The activity of glycogen phosphorylase has been studied at intervals in all the 3 types of eggs to examine whether the enzyme activity can always account for changes in glycogen levels and to see how the activity of the enzyme differs in diapause and non-diapause eggs.

2. Material and methods

2.1 *Materials*

Bivoltine (NB_4D_2) and multivoltine (Pure Mysore) silkworm races were maintained under standard conditions. Diapause eggs of the bivoltines and non-diapause eggs of multivoltines were used for the experiments. Loose eggs were prepared on polythene sheets and kept at 25°C with a relative humidity of 75%.

2.2 *Breaking of diapause*

For breaking diapause, 20 h old diapause eggs were treated with HCl solution of specific gravity 1·075 at 46·1°C for 3–4 min, washed thoroughly with water and dried. The eggs were then kept at 25° ± 2°C.

2.3 *Determination of glycogen in eggs*

Glycogen was determined by the method of Hassid and Abraham (1957) with slight modification.

2.4 *Enzyme preparation*

A 10% (w/v) homogenate of the eggs were prepared in ice cold distilled water with a glass homogenizer fitted with teflon pestle. The homogenate was filtered through a cotton pad and centrifuged at 5500 g for 15 min at 0°C. The supernatant was filtered through Whatman No. 1 filter paper and the resultant filtrate was used as the enzyme source.

2.5 *Determination of enzyme activity*

The activity of phosphorylase was assayed according to the procedure of Cori *et al* (1973). The inorganic phosphate was estimated by Fiske and Subba Row (1925) method. The enzyme activity is expressed as μ mol P_i/min/mg protein. Protein concentration was determined according to the method of Lowry *et al* (1951) using Bovine serum albumin as standards.

All the above experiments were conducted in 7–8 samples and the mean values expressed along with the standard deviation.

3. Results

3.1 *Studies on glycogen levels*

At the time of egg laying, the level of glycogen was found to be more in diapause eggs than in non-diapause eggs (figures 1 and 2).

In non-diapause eggs, the glycogen level decreased very gradually during the first 3 days following oviposition and on the 3rd day, the glycogen content of the eggs was not much different from that of the newly laid eggs. Following this period, there was a more significant decrease and glycogen decreased to 60% of the initial content around the 9th day.

In diapause eggs after acid treatment, with the onset of development glycogen level followed a pattern very similar to that observed in non-diapause eggs (figure 2). In diapause eggs, a pattern altogether different from the above two types was seen (figure 3). The glycogen content started decreasing markedly and suddenly 1 day after oviposition and it was reduced to 25% of the initial level within 4 days after oviposition. The glycogen level decreased to about 17% of the initial level by the 10th day and it remained at this level for a long time (figure 4).

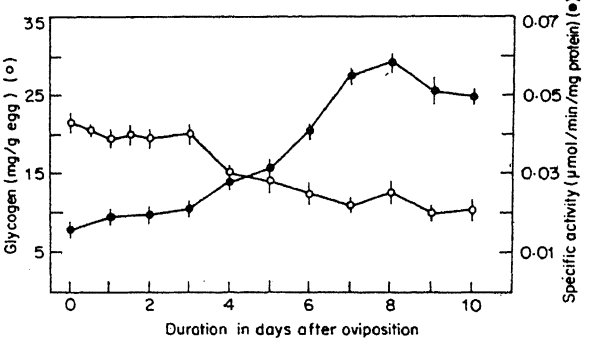

Figure 1. Changes in the glycogen level and glycogen phosphorylase activity during embryonic development in non-diapause eggs.

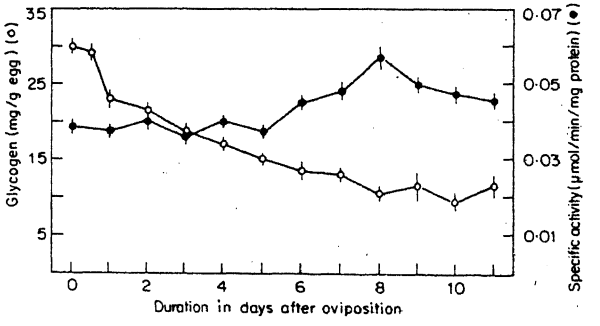

Figure 2. Changes in the glycogen level and the glycogen phosphorylase activity during embryonic development in bivoltine eggs after acid treatment.

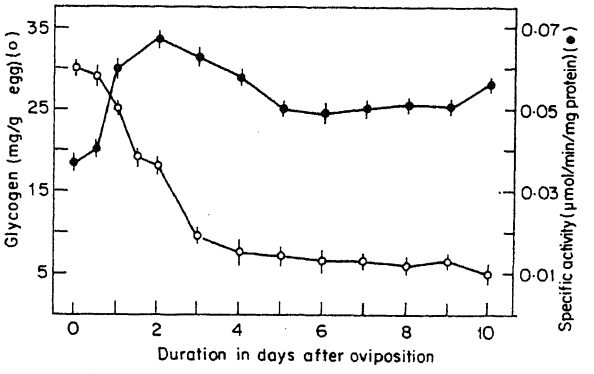

Figure 3. Changes in the glycogen level and the glycogen phosphorylase activity during diapause in bivoltine eggs.

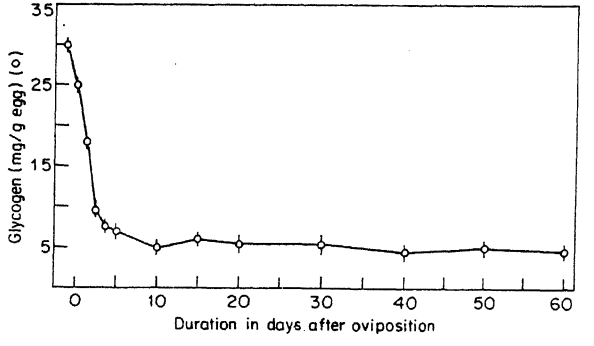

Figure 4. Changes in the glycogen content of the bivoltine eggs during diapause.

3.2 *Studies on phosphorylase activity*

In non-diapause eggs, the phosphorylase activity was low to start with after egg laying (figure 1). The level started increasing about 3 days after oviposition and was highest at the time of hatching.

In diapause eggs after acid treatment, the total phosphorylase activity was comparable to that observed in non-diapause eggs. There was an increase in the level of activity about 5 days after acid treatment and a maximum level was obtained around the 8th day after oviposition (figure 2).

In diapause eggs, the level of activity soon after oviposition was slightly higher than that observed in non-diapause eggs. But the activity suddenly increased 12 h after oviposition to reach a peak on the 2nd day following which the activity decreased to 74% of the peak level which was more than the initial level and around this increased level it was maintained over a long period (figure 3).

4. Discussion

It is evident from our studies that the glycogen content of the diapause eggs at the time of oviposition is much higher than that of non-diapause eggs. This may be an

adaptive phenomenon. Because, in non-diapause eggs the embryonic development proceeds and completes in about 10 days while in diapause eggs, considerably long period precedes the development and a demand for energy can be expected to occur during diapause even though to a smaller degree compared to that during embryonic development.

The glycogen level gradually decreased in non-diapause eggs and similar decrease in glycogen level was also observed in diapause eggs after acid treatment, showing that glycogen may be an important source of energy during growth and differentiation in silkworm eggs. During the early phase of embryonic development, the non-diapause eggs provide a marked contrast to diapause eggs. While in non-diapause eggs, the glycogen level decreases very gradually, in diapause eggs rapid fall in glycogen level is observed. Thus, in diapause eggs though growth and development are temporarily suspended, glycogen is being broken down for some purpose. This marked decrease in glycogen level also coincides with the onset of diapause. These observations agree with those of Chino (1957) and Yamashita *et al* (1975).

The activity of the enzyme phosphorylase shows a reciprocal relationship with the level of glycogen in all the 3 types of eggs. In diapause eggs phosphorylase activity reaches a very high level 2 days after oviposition explaining the rapid rate of glycogen break down associated with the initiation of diapause.

Newsholme and Start (1973) have shown that glycogen phosphorylase plays an important role in the metabolism of glycogen in many species of animals. Our studies also indicate that glycogen phosphorylase plays an important role in the utilization of glycogen at the initiation of diapause.

The pattern exhibited by phosphorylase activity, wherein it increases accompanied by decrease in glycogen level seems to be a general pattern in silkworms since the same was also reported in the eggs of *Philosamia ricini* (Pant and Nautiyal 1974). Interestingly, when non-diapause eggs of silkworm were subjected to anoxia, the glycogen phosphorylase activity was found to be abruptly elevated (Yamashita *et al* 1981), showing that there is always a rapid break down of glycogen associated with the onset of diapause and that phosphorylase acts as a key enzyme involved in this glycogen utilisation. Hence, it is suggested that glycogen phosphorylase plays a key role in regenerating the glycosyl units either as a source of energy or as building blocks for biosynthetic reactions in silkworm eggs during the embryonic development and during diapause.

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A shift in metabolic pathway of *Sarotherodon mossambicus* (Peters) exposed to thiodon (endosulfan)

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Abstract. Total oxygen consumption, glycogen content and succinic dehydrogenase levels in liver, muscle and heart in normal and thiodon-exposed (to sub-lethal concentration for 48 h and to lethal concentration for 6 h) *Sarotherodon mossambicus* (Peters) were studied. While oxygen consumption of the fish dropped to 43 and 35% respectively under sub-lethal and lethal exposures, the magnitude of decrease in tissue glycogen content as well as tissue succinic dehydrogenase level followed a uniform order of liver > muscle > heart in both the exposures. Reduction in oxygen uptake and tissue glycogen levels were indicative of the onset of hypoxia involving utilization of stored glycogen by the fish under thiodon exposure. The inhibition of succinic dehydrogenase levels in the 3 tissues of thiodon-exposed *Sarotherodon mossambicus*, while indicating the impairment of aerobic metabolism, is also suggestive of a shift from aerobic to anaerobic metabolism in the fish under pollution stress.

Keywords. *Sarotherodon mossambicus*; thiodon; succinic dehydrogenase; aerobic metabolism; anaerobic metabolism.

1. Introduction

Pesticides are known to affect the oxygen consumption and metabolic pathways of non-target species like fish in freshwater ecosystems. Effect of pesticides on the oxygen consumption of fish has been studied in *Mystus vittatus* (Reddy and Gomathy 1977) and *Colisa lalia* (Reddy *et al* 1977; Uthaman 1977). Utilization of stored glycogen by the fish under pesticide stress was reported in *Heteropneustes fossilis* (Qayyam and Shaffi 1977), *Labeo rohita*, *Ophiocephalus punctatus* and *Clarias batrachus* (Shaffi 1979), *Channa punctatus* (Shah and Dubale 1983) and *Tilapia mossambica* (Vasanthi 1983) exposed to different pesticides. Inhibition of succinic dehydrogenase (SDH) (EC 1·3·99·1) enzyme activity has also been uniformly observed in *Tilapia mossambica* (Koundinya and Ramamurthi 1978), *Channa striatus* (Natarajan 1981), *H. fossilis* (Dubale and Mohini Awasthi 1982) and *T. mossambica* (Kabeer *et al* 1983) under pesticide-exposed conditions. While the effect of pesticide on oxygen consumption, stored glycogen content and SDH activity levels of tissues were studied in different fishes exposed to different pesticides (providing only fragmentory informations), the present paper provides a comprehensive information on the effects of a particular pesticide on the metabolic pathway of a particular fish by studying total oxygen uptake, stored glycogen levels and SDH activity levels of liver, muscle and heart of normal *S. mossambicus* (Peters) and of those exposed to an organochlorine pesticide, thiodon (endosulfan).

2. Materials and methods

Thiodon, an organochlorine as well as organic sulphite pesticide, is in wide usage in

the eradication of cotton pest in Coimbatore District. The pesticide washes from cotton fields were observed to pollute 4 major lakes in and around Coimbatore City which are the chief sources of inland fisheries. *S. mossambicus*, locally called as Jilebi Kendai, is the major fish variety available from these lakes throughout the year.

Samples of *S. mossambicus* (5–9 g), obtained from 4 major lakes in and around Coimbatore City, were maintained in cement tanks at 28 ± 1°C and fed with cooked rice regularly. Feeding was discontinued one day before the experiment.

A stock solution of technical grade thiodon (supplied as 'Hochest' product by Parry Limited, Bombay) in acetone was used to prepare different concentrations used in the static bioassay study. Sub-lethal (0·001 ppm) and lethal (0·005 ppm) concentrations were determined employing repeated exposure experiments and Probit analysis method (Finney 1964).

Fishes were exposed (in glass jars of 3 l capacity) to 0·001 ppm thiodon for 48 h and to 0·005 ppm thiodon for 6 h. The pesticide water containing 0·001 ppm thiodon was renewed every 12 h to maintain the dissolved oxygen content and pesticide concentration constant throughout the period of exposure. Throughout this investigation, control fishes were also treated with 0·25 ml acetone/l which formed the largest aliquot of stock used in the pesticide water.

2.1 *Measurement of oxygen consumption*

Oxygen consumption of control and thiodon-exposed (TE) fish was measured using a simple respiratory chamber by measuring the loss of oxygen content (due to the respiration of the fish) of water in the respiratory chamber. Oxygen content of water sample was estimated using Winkler's method (Welsh and Smith 1960). The oxygen consumption of the fish was expressed in ml/kg/h.

Weighed samples of liver, muscle and heart tissues were dissected out from the control and TE fish by keeping the stunned (by a blow on the head) fish in an iced trough and used for estimation of glycogen content and SDH activity levels.

2.2 *Estimation of glycogen content*

The glycogen content of liver, muscle and heart was estimated by employing the method of Kemp and Kits (1954). The weighed tissue sample was homogenized in 5 ml of 80% methanol and centrifuged. The residue was collected and treated with 5 ml of 5% tricarboxylic acid (TCA) (w/v). The mixture was boiled for 5 min in a water bath, again made upto 5 ml by adding 5% TCA and centrifuged. To 2 ml of the supernatant, 6 ml of concentrated sulphuric acid (Analar grade) was added and heated in a boiling water bath for exactly 6·5 min. The final mixture was cooled and the colorimetric reading was obtained at 530 μ using a photoelectric colorimeter (Erma, AE–11 model, Japan). Using the colorimetric readings, glycogen values were obtained from a standard graph already prepared using known glucose standard solutions. The glycogen contents of tissues were expressed in mg/g of tissue.

2.3 *Estimation of SDH activity*

SDH enzyme activity level of the 3 tissues were estimated using the method of

Nachlas *et al* (1960). For the assay of SDH activity, a 5% homogenate of the tissue was prepared with 0·25 M cold sucrose solution. The homogenate was centrifuged at 3000 rpm for 15 min to remove the cell debris. Enzyme reaction mixture was prepared with 0·1 ml of 0·5 M sodium succinate; 1·0 ml of phosphate buffer (pH 7·4); 1·0 ml of INT solution [(2-*p*-iodophenyl)-(3-*p*-nitrophenyl-5-phenyl tetrasolin chloride)]; 0·5 ml of 5% homogenate supernatant and 0·4 ml of distilled water. The reaction was stopped after 30 min of incubation at 37°C by adding 6·0 ml of acetic acid. The formazon formed was extracted into 6·0 ml of toluene after the mixture was kept in freeze for 12 h. The optical density (OD) of pink colour formed was read at 495 μ using a photoelectric colorimeter (Erma, AE-11 model, Japan). A reagent blank (to set the colorimeter) was prepared as above except 0·5 ml of supernatant. Instead, 0·5 ml of distilled water was added. From a standard graph of formazon formed, μmol of formazon formed in the enzyme reaction mixture was obtained. SDH activity levels of the tissues were given as μmol formazon/mg tissue/h.

Changes in oxygen consumption, tissue glycogen content and SDH activity levels of TE fish from that of control fish were expressed in percentages and tested for statistical significance using Student's '*t*' test.

3. Results

Oxygen consumption of control and TE *S. mossambicus* are presented in table 1. Table 2 provides the glycogen content in liver, muscle and heart of control and TE fish. Levels of SDH activity in liver, muscle and heart of control and TE fish are given in table 3.

S. mossambicus showed a 43% reduction in oxygen consumption when exposed to sub-lethal concentration (0·001 ppm) of thiodon for 48 h. When exposed to lethal concentration (0·005 ppm) of thiodon for 6 h, the fish also showed a significant drop in oxygen uptake to the tune of 35% (table 1).

Of the 3 tissues examined, liver appears to be the major site of stored glycogen content. Exposure of *S. mossambicus* to thiodon concentrations caused significant drop in glycogen content of all the 3 tissues studied. Under sub-lethal exposure, the drop in glycogen content of liver, muscle and heart were 78, 73 and 67% respectively. When exposed to lethal concentration of thiodon, the liver showed a minimum 9% drop in its glycogen content which was statistically not significant. This indicates

Table 1. Oxygen uptake of control and thiodon-exposed *S. mossambicus* expressed in ml/kg/h ± S.E.

	Thiodon-exposed (ppm)	
Control	0·001	0·005
210·00[a] ± 20·12	118·90[b] ± 16·38 (−43) $P < 0·01$	137·20[c] ± 17·91 (−35) $P < 0·05$

[a]Mean value of 6 observations; [b]Mean value of 5 observations; [c]Mean value of 7 observations.

'−' denotes % decrease from control level. (% Changes from control levels are given in parentheses).

Table 2. Glycogen levels in liver, muscle and heart of control and thiodon-exposed *S. mossambicus* expressed in mg/g of tissue ± S.E.

Tissues	Control	Thiodon-exposed (ppm)	
		0·001	0·005
Liver	4·69 ± 0·38	1·05 ± 0·10 (−78) $P < 0·01$	4·28 ± 0·66 (−9) NS
Muscle	2·33 ± 0·04	0·63 ± 0·10 (−73) $P < 0·05$	0·48 ± 0·05 (−79) $P < 0·01$
Heart	2·40 ± 0·10	0·79 ± 0·12 (−67) $P < 0·01$	0·55 ± 0·05 (−77) $P < 0·01$

NS, Not significant statistically ($P > 0·05$).
'−' denotes % decrease from control level.
Values are means of 7 observations (% changes from control levels are given in parentheses).

Table 3. SDH activity in liver, muscle and heart of control and thiodon-exposed *S. mossambicus*, expressed in μmol formazon/mg tissue/h.

Tissues	Control	Thiodon-exposed (ppm)	
		0·001	0·005
Liver	0·57 ± 0·17	0·18 ± 0·02 (−68) $P < 0·01$	0·37 ± 0·14 (−35) $P < 0·01$
Muscle	0·19 ± 0·03	0·17 ± 0·01 (−11) $P < 0·01$	0·17 ± 0·03 (−11) $P < 0·01$
Heart	0·29 ± 0·02	0·26 ± 0·06 (−10) $P < 0·01$	0·28 ± 0·08 (−4) NS

NS, Not significant statistically ($P > 0·05$).
'−' denotes % decrease from control level.
Values are means of 6 observations ± S.E. (Changes from control levels are given in parentheses).

that utilization of stored liver glycogen occurs only upon prolonged exposure. On the other hand, muscle and heart tissues showed increased magnitudes of drop in glycogen contents to about 79 and 77% respectively (table 2) which implies that utilization of stored glycogen content increased with increase in concentration of pesticide irrespective of the period of exposure. This variation in the drop in glycogen content of the 3 tissues indicates that different tissues behave differently under pesticide exposure.

The data on tissue SDH activity levels clearly indicate an inhibition of SDH activity to about 68, 11 and 10% respectively in liver, muscle and heart tissues of

S. mossambicus exposed to sub-lethal concentration of thiodon (table 3). However, exposure of fishes to lethal concentration of thiodon elicited only a lesser magnitude of drop in SDH activity level in the liver tissue (35%). Muscle tissue showed a similar drop in SDH level as that of 0·001 ppm TE fishes under lethal exposure (11%). The non-significant minimum drop (4%) in SDH activity level of heart tissue under lethal exposure of the fish is noteworthy for discussion (table 3).

4. Discussion

A perusal of table 1 clearly indicates that exposure of fish to sub-lethal and lethal concentrations of thiodon caused a definite reduction in oxygen uptake of the fish. A similar depression in oxygen uptake has been reported in *C. lalia* (Reddy *et al* 1977; Uthaman 1977) and *Mystus vittatus* (Reddy and Gomathy 1977) exposed to different pesticides. The decreased oxygen uptake in TE *S. mossambicus* (in the present study) could be suggested as a sequel to gill damage upon pesticide exposure as reported in *C. striatus* (Natarajan 1981) or due to hypochromic microcytic anaemia caused due to pesticide toxicity as reported in *S. mossambicus* (Koundinya and Ramamurthi 1979), *C. striatus* (Natarajan 1981) and *C. batrachus* (Goel *et al* 1984). The drop in oxygen uptake of TE *S. mossambicus*, observed in the present investigation, indicates the onset of a severe hypoxia in the fish following pesticide exposure which will trigger on some biochemical changes in different tissues of the fish body.

Anoxia or hypoxia is known to increase carbohydrate consumption (Dezwaan and Zandee 1972). The unequivocal reductions in stored glycogen content in liver, muscle and heart of 0·001 ppm TE fish and in muscle and heart of 0·005 ppm TE fish indicate the utilization of stored glycogen possibly through anaerobic glycolysis to meet the energy requirement under hypoxia caused due to pesticide stress. A similar reduction in stored tissue glycogen content has been reported in *H. fossilis* exposed to mercuric nitrate (Qayyam and Shaffi 1977), *L. rohita*, *O. punctatus* and *C. batrachus* following copper sulphate intoxication (Shaffi 1978), *A. scandens* exposed to zinc sulphate (Natarajan 1981), *T. mossambica* exposed to lindane (Vasanthi 1983) and methyl parathion (Siva Prasada Rao and Ramana Rao 1979) and *C. punctatus* exposed to malathion (Shah and Dubale 1983).

The data on tissue SDH activity levels, obtained in the present study (table 3), clearly indicate an inhibition of SDH activity in liver, muscle and heart of 0·001 ppm TE fish and in liver and muscle of 0·005 ppm TE fish. A similar drop in SDH activity levels of different tissues has also been reported in *S. mossambicus* exposed to lindane (Lakshmi 1984), *C. striatus* exposed to metasystox (Natarajan 1981), *H. fossilis* (Dubale and Mohini Awasthi 1982) and *T. mossambica* under malathion stress (Kabeer *et al* 1983). SDH being a key enzyme in TCA cycle, its inhibition under toxic condition, as observed in liver, muscle and heart of *S. mossambicus* exposed to sub-lethal concentration of thiodon (in the present study) and also reported in other fishes exposed to different pesticides could be considered as indication of the fish showing a shift towards anaerobic metabolism (utilizing stored metabolites like glycogen) under toxic conditions. Based on SDH and LDH studies, Koundinya and Ramamurthi (1978) suggested the operation of anaerobic glycolysis in different tissues of *T. mossambica* exposed to sumithion. Exposure of fish to pesticide, thus, causes impairment of an important enzyme system (SDH activity) besides other toxic effects.

The observed insignificant 4% drop in SDH activity level of heart tissue under lethal exposure of the fish, inspite of a significant 77% reduction in stored heart glycogen content under similar exposure, indicates a continued operation of aerobic breakdown of glycogen in the heart tissue to meet energy demand during early periods of exposure. This further indicates that the heart is aerobic possibly due to increased oxygen supply to the heart in order to prevent the onset of lactic acidosis in the heart tissue which will occur normally during hypoxic conditions. Thus the heart tissue is found adaptive in maintaining itself physiologically fit for effective cardiac function during early periods of pesticide exposure.

In conclusion, it could be stated that exposure of *S. mossambicus* to thiodon elicited a severe hypoxia resulting in the utilization of stored glycogen by way of anaerobic glycolysis to meet the energy demand during pesticide stress. The inhibition of SDH activity levels in different tissues of TE *S. mossambicus*, while suggesting the failure of aerobic metabolic pathway, also hints upon the possibility of a shift from aerobic to anaerobic mode of energy metabolism in tissues.

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Aspects of culture: *Acanthamoeba astronyxis* (Ray and Hayes 1954) from Bay of Bengal coasts, India

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Abstract. *Acanthamoeba astronyxis* strains, isolated from intertidal belts of Sagar Island (21°30′ to 21°50′ N and 88°04′ to 88°08′ E) facing Bay of Bengal, were cultured in saline distilled water agar medium supplemented with *Escherichia coli* as food bacterium. Population growth in bay water agar, cerophyl bay water agar and soil extract distilled water agar were compared with special reference to devised saline distilled water agar medium. The overall growth showed little variation over ranges of media salinity (5–30‰). However, greater values were encountered in media of intermediate range. Isolate from mangrove litter-soil of highest high tide belt (strains) grew more efficiently in cerophyl bay water agar and soil extract distilled water agar media while mid-littoral and lowest low tide isolates preferred bay water agar and saline distilled water agar media more.

Keywords. *Acanthamoeba astronyxis*; growth comparison.

1. Introduction

Free-living small naked amoebae are ubiquitous in their distribution and Singh (1973) contends that soil constitutes a reservoir for them to disseminate in other environments. Bovee and Sawyer (1979) put forward the idea that their wide distribution in coastal and estuarine benthic habitats is owing to wave action and ocean currents.

The maintenance of a continuous culture for amoebae is a prerequisite for any planned study of amoeba biology as conventional standard methods are lacking to preserve them in their typical locomotive forms. Therefore, samples must be cultured to determine their growth under varying conditions. Several workers have proposed and successfully used different media to culture amoebae isolated from all probable sources of natural environments (Adams 1959; Bovee and Sawyer 1979; Chang 1958; Davis *et al* 1978; Dixon 1937; Fulton 1970; Menapace *et al* 1975; Page 1967, 1970; Prescott 1956; Prescott and James 1955; Sawyer 1971, 1975; Singh 1945, 1955).

One of the most predominant genera of naked amoebae, *Acanthamoeba* is relatively tolerant of high osmolarities and has often been found in salt water (Page 1981). Species belonging to this genus have repeatedly been isolated as a predominant microbenthic component from Gangetic estuary facing bay (Bhattacharya *et al* 1985; Choudhury and Ghosh 1985; Ghosh and Choudhury 1985a, b, c, 1986; Ghosh *et al* 1985). The present investigation has been envisaged to culture and study the growth of *A. astronyxis* strains, isolated from intertidal flats of bay coast in media of different salinities and also of different ingredients.

2. Materials and methods

2.1 *Sampling*

Samples upto 3 cm depth from different littoral belt areas during the monsoonal period (July to October) were collected aseptically and transported to the laboratory and were processed soonafter.

The highest high tide samples were typically litter-soils with decaying leaves of *Excoecaria agallocha* L., *Avicennia* sp., *Phoenix paludosa* Roxb, *Acanthus ilicifolius* L. etc. The mid-littoral belt was characterized by hard loamy clay with dead and decaying roots of the recently deforested 'Mangal'. The lowest low tide samples were typically sand-silt type.

2.2 *Isolation and identification of A. astronyxis strains*

Samples from the 3 zones were inoculated on *Escherichia coli* patch in respective soil extract distilled water agar (SEDWA) petri dishes. Establishment of *A. astronyxis* clones corroborated the observations of Singh and Hanumaiah (1979).

2.3 *Food source*

Laboratory maintained *E. coli* was used as food. The ingredients of the culture media were: 1 l of glass distilled water (pH 6·4), 20 g NaCl, 20 g Bacto Agar (Difco Labs., USA), 2·60 g Na_2HPO_4, 2 g glucose and 20 g Bacto Tryptone (Difco Labs., USA).

2.4 *The supporting. media*

2.4a *Media of different salinity gradients*: Saline distilled water agar (SDWA) media: Each of the 3 sets of experiments require 6 media of different salinity strengths ranging from 5 – 30‰ (w/v) at 5‰ intervals. To each of the 6 containers, 1 l of glass distilled water was taken and pH was adjusted to 8·3 which was found to be the approximate mean value of bay water during monsoon. NaCl of the quantities 5, 10, 15, 20, 25 and 30 g were then added separately to each container along with 15 g agar. They were boiled, autoclaved and dispensed in sterile 7·5 cm dia petri dishes in 3 replicates for each medium.

2.4b *Bay water agar (BWA) medium*: 15 g of agar was added to 1 l of filtered bay water, boiled and autoclaved. pH was adjusted as before.

2.4c *Cerophyl bay water agar (CBWA) medium*: This was prepared after Page (1970) with minor modifications. 1 g of cerophyl (Cerophyl Lab. Inc., USA) was boiled in 1 l of filtered bay water, filtered thrice through glass wool. 15 g of agar was then added, boiled and autoclaved. pH was adjusted as before.

2.4d *Soil extract distilled water agar (SEDWA) medium*: This was prepared after

Singh (1975) with minor modifications. 400 g of littoral belt samples were boiled in 1 l of glass distilled water for 30 min, extracts were decanted, filtered and finally made to 1 l. 15 g of agar was then added to each, boiled and autoclaved. pH was adjusted as before.

2.5 *Modified Page's amoeba saline (PAS)*

For organisms growing in SDWA media the chemical ingredients of the PAS were same as in Page (1966) except NaCl which was adjusted as per the salinity of the respective fluid base of SDWA medium. To 1 l of glass distilled water the following components were added: $MgSO_4.7H_2O$, 0·004 g; $CaCl_2.2H_2O$, 0·004 g; Na_2HPO_4, 0·142 g and KH_2PO_4, 0·136 g. For the organisms from all other media, chemicals other than NaCl were added to respective fluid bases.

2.6 *Experimental methodology*

E. coli from young cultures were used as food. They were suspended in as many tubes as the sets of experiments containing respective sterile modified PAS. After centrifugation and triple wash, the pellets were resuspended to make a final wet weight suspension of 0·05 g/ml.

To 3 replicate plates for each experiment, *E. coli* suspensions were added, so that each received about 0·01 g wet weight of microorganisms. Excess fluid was allowed to absorb. Cyst from each clonal culture was then inoculated on media plates and kept in BOD incubator at 25 ± 1°C. Efficacy of the media used, to support the growth of *A. astronyxis* strains under study was assessed as follows.

The medium was considered to be non-efficient if the mean number of organisms in culture of 6 fields of 2 replicate plates is less than 25 per microscopic field of 100 × after desired periods of 3, 7, 14, 21, 28, 35 and 42 days. One moderately efficient medium would show a value ranging between 25 and 100 under such condition. While a highly efficient medium exhibits more than 100 organisms per field. The overall growth was enumerated after 100 days of culture using haemocytometer (mean of 3 replicate plates). Some organisms may have been lost while harvesting.

3. Results

The efficiency of different SDWA media to support the growth of *A. astronyxis* strains is shown in figure 1. Within 3 days in media having salinities 10, 15 and 25‰ the amoebae showed moderately efficient growth for lowest low tide (LL) strains while the 20‰ medium exhibited highly efficient growth. Poor growth for the same strain was observed in 5 and 30‰ salinity media.

Mid-littoral (ML) strains showed very efficient growth in media ranging from 10–20‰ salinities. Moderate growth was observed in media of 5 and 25‰ salinities. Within the same period poor growth was encountered in 30‰ saline medium. All the media except 30‰ promoted efficient growth within 3 days for high tide belt (HH) strains. 30‰ saline medium showed poor growth during this period. Within 7 days, most of the media promoted highly efficient growth. The exceptions were 5‰

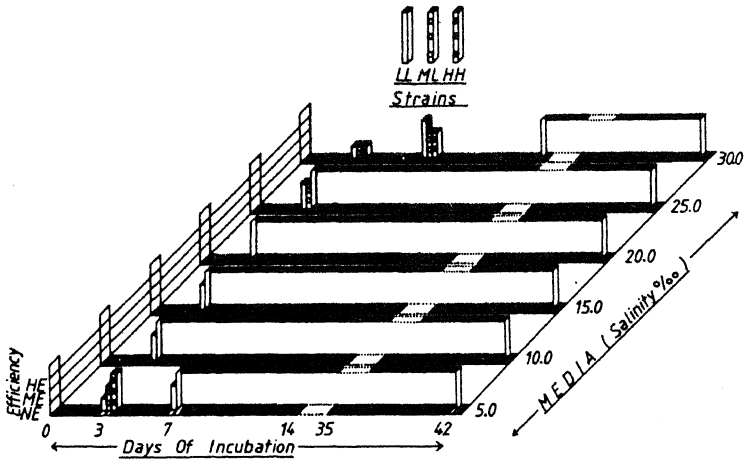

Figure 1. Efficiency of the media of different salinity gradients to support growth of *A. astronyxis* strains. NE (Non-efficient media): Average count in 6 fields (100×) of 2 replicate plates upto 25 organisms; ME (Moderately efficient media): This value lies between 25 and 100; HE (Highly efficient media): More than 100 organisms.

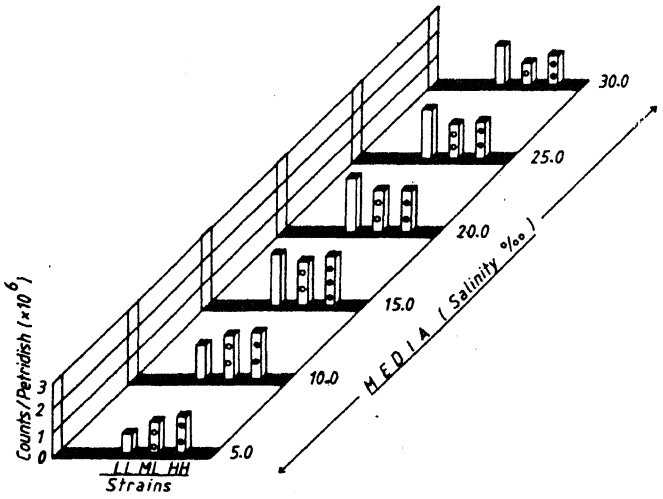

Figure 2. Overall growth of *A. astronyxis* strains after 100 days of culture in media of different salinity strengths.

medium for LL strains, and 30‰ medium for both ML and HH strains which show moderately efficient growth. Then onwards all the media supported highly efficient growth for all the investigated strains. The growth efficiency in 4 selected media viz SDWA (20‰), BWA, CBWA and SEDWA were also observed. It is evident that all the media initiated highly efficient growth within 3 days. Two bar diagrams are shown in figures 2 and 3. The former depicts growth in SDWA media. Average mean values range between 0·2 to $1{\cdot}3 \times 10^6$. Media of intermediate salinities supported maximum growth. The latter presents growth in 4 selected media. Average growth

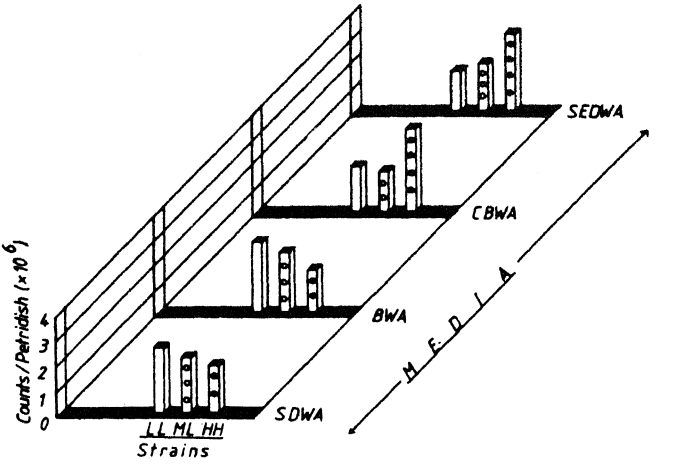

Figure 3. Growth of *A. astronyxis* strains in 4 selected media: SDWA, BWA, CBWA and SEDWA.

ranges between 1·1 to 2·1 × 10^6. Maximum values for HH strain were obtained in CBWA and SEDWA media while ML and LL strains grew better in BWA and SDWA media.

4. Discussion

Amoebae are frequent in marine neuston (Sieburth *et al* 1976; Sieburth 1979) and benthic situations (Sawyer 1980) and are important epibiotic fauna preying upon phytoplankton and bacteria (Bamforth 1981). Their importance in the oceanic ecosystem has only recently begun to be assessed as Bovee and Sawyer (1979) point out that bactivorous and the scavenging forms probably maintain the cleanliness of shallow inshore water and the surface water of open ocean. Page (1980) points to the paucity of data concerning the ecological role of naked amoebae as marine and estuarine benthos.

The media described in the present investigation have been used to culture amoebae isolated from diversified samples, including typical litter-soil, hard loamy, silt-clay and the sand types. Comparison of population growth shows that they do not encyst on challenge with 'salt stress' but adjust and quickly cope up with the new environment. These observations are in accord with a general impression that intertidal fauna, experiencing estuarine fluctuating environment are in need to acquire a certain degree of euryhalinity as an insurance against such conditions. A harmonious ecological balance thus is required between environmental conditions and the tolerance of organisms to variations in one or more of the said conditions to successfully colonize and maintain a population.

After studying in detail the growth of *A. astronyxis* strains in media of different components and proportions, we would like to propose finally that, since little variations in growth were exhibited by different media, those of use should initiate highly efficient growth within the shortest period. Moreover, it would certainly be a

good practice to avoid media from either of the extremities, atleast for typical deltaic samples, like the ones in the present investigation.

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Development and survival of *Mythimna separata* (Wlk.) on some host plants during winter at Hisar

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Abstract. Survival and post embryonic development of the oriental armyworm, *Mythimna separata* (Wlk.) was studied on 5 host plants, viz sorghum, maize, sugarcane, Johnson grass and napier grass during winter months (15 November to 7 March) under laboratory conditions. Variations in different parameters like larval survival (10·3–60·3%), size of larvae (7·33–29·67 mm), larval weight (25·5–493·1 mg for 26 days old larva), size of pupa (15·0–16·49 mm), pupal weight (184·9–249·5 mg for 2–4 days old pupa), pupation (3·3–38·9%), developmental period (74·8–109·8 days) and moth emergence (3·4–50·0%) were significant on different foods. Low temperature and humidity conditions during experimental period resulted in complete mortality of female pupae reared on napier grass, Johnson grass and maize whereas in case of males 60–100% moths were crippled winged. However, adults emerged on sorghum and sugarcane foods were normal except 28·7% males in sorghum were crippled. Total growth index was also higher on sugarcane (0·45) and sorghum (0·24) indicating their suitability in carrying over of winter pest population.

Keywords. Armyworm; *Mythimna separata*; host plant; growth index.

1. Introduction

The oriental armyworm, *Mythimna separata* (Wlk.) (Lepidoptera: Noctuidae) is a ubiquitous pest of cereal crops and grasses in Asia and Australia. About 16 serious outbreaks of the pest have been recorded in India from 1924–1981 (Sharma and Davies 1983). In northern part of the country it has been reported to cause considerable damage intermittently to many graminaceous crops (Chaudhary and Ramzan 1967; Sarup *et al* 1969; Verma and Khurana 1971; Bindra and Singh 1973; Butter *et al* 1979; Chaudhary and Singh 1980; Singh and Manchanda 1981). This armyworm in recent years has started assuming serious proportions quite frequently and little information is available on the extent of survival of winter generation of this pest on various host plants. Keeping this in view, the present investigations were carried out.

2. Material and methods

Studies were carried out on 5 host plants, viz Sorghum (*Sorghum bicolor* (L.) Moench), maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), Johnson grass (*Sorghum halepense* (L.) Pers) and napier grass (*Pennisetum purpureum* Schumach) from 15 November 1984 to first week of March 1985 under fluctuating laboratory conditions at the University. Freshly hatched larvae of armyworm were fed on fresh young leaves of these grasses. First instar larvae were taken from the mass culture maintained in laboratory on leaves of pearl millet and maize. Small pieces of fresh leaves of each grass were put in glass jars (20 × 15 cm). In each jar, 100 freshly

hatched larvae for each test plant in 3 replications were released. These jars were covered with muslin cloth and placed under laboratory conditions. Temperature and humidity prevailing in the laboratory were recorded daily with the help of maximum–minimum, dry and wet bulb thermometers, respectively. The fresh food was provided daily. Various developmental parameters, viz larval survival, durations, size and weight; pupation, pupal weight, size and durations; pre-pupal duration, moth emergence, adult longevity etc were recorded for each food separately. Sexing was done in pupal stage as suggested by Avasthy and Chaudhary (1964). The growth indices on different foods were calculated by the following formulae (Sharma *et al* 1982).

$$\text{Larval growth index} = \frac{\text{Pupation (\%)}}{\text{Mean larval period (days)}}$$

$$\text{Total growth index} = \frac{\text{Moth emergence (\%)}}{\text{Mean total developmental period (days)}}$$

3. Results and discussion

From the data summarised in table 1, larval survival when recorded after 5 days of release, ranged from 74·7–97·0% on different foods without any significant difference among them. Thereafter, it decreased gradually except in case of sugarcane where only 10·3% survival was observed on 22nd day of feeding. Sugarcane though a potential host plant of armyworm (Butani 1955; Chaudhary and Ramzan 1967), appeared to be less acceptable by first instar larvae probably due to hard leaf tissues. Kalode *et al* (1971) also reported 100% mortality of young larvae of *Cirphis compta* Moore on sugarcane leaves.

Maximum (60·3%) larvae survived on napier followed by Johnson grass (54·3%) and sorghum (48·7%) without any significant difference among them. On maize leaves, only 36·7% larvae could survive. Under varying conditions of temperature and humidity larval survival of this pest on maize had been reported to vary from 12·0–60·0% (Bindra and Singh 1973; Dhaliwal and Bains 1978; Alam and Khatri 1980).

Table 1. Effect of feeding on different host plants on the larval survival, size and weight of *M. separata* under laboratory conditions.

	Larval survival (%) (days)				Size of larva (mm) (20 days old)	Larval weight (mg) (days)		
Test plant	5	10	15	22		14	23	26
Napier grass	97·0	77·7	71·3	60·3	29·67	56·3	358·5	493·1
Sorghum	74·7	56·0	49·7	48·7	25·49	54·0	339·5	482·5
Johnson grass	76·3	66·0	59·7	54·3	25·00	59·3	284·0	306·6
Maize	94·0	61·7	47·7	36·7	22·33	36·9	254·0	344·2
Sugarcane	90·7	27·7	17·3	10·3	7·33	5·5	25·4	25·5
Average	86·5	57·8	49·1	42·0	21·96	42·4	252·3	330·4
SEM ±	9·8	4·8	5·5	5·9	0·80	4·6	19·4	62·9
C.D. at 5%	NS	11·1	12·7	13·7	1·86	10·6	44·8	128·1

Mean temperature = 18·6 ± 3·2°C, Mean relative humidity = 55 ± 5%.
*Based on 300 larvae.

Size of the larvae compared after 20 days of release ranged from 7·33 (sugarcane) to 29·67 mm (napier) indicating the slow growth of larvae on sugarcane. Larval weights after 14 days of feeding showed considerable differences on different foods. It ranged from 5·5 mg on sugarcane to 56·3 mg on napier grass. After 26 days of feeding, the range (25·5–493·1 mg) was still wider. Larvae fed on napier gained maximum weight (493·1 mg) followed by sorghum (482·5 mg) without any significant difference between them. On maize and Johnson grass, larva weighed 344·2 and 306·6 mg, respectively and were also significantly heavier than sugarcane (25·5 mg).

Pupal size on different foods ranged from 15·0–16·91 mm (table 2). Avasthy and Chaudhary (1965) and Alam and Khatri (1980) also reported similar pupal sizes on sorghum, maize and sugarcane. Pupal weight of 2–4 days old pupa varied between 184·9 mg (sugarcane) and 249·5 mg (sorghum) at 18·2 ± 3·9°C and 63 ± 4·0% relative humidity. However, Tripathi *et al* (1982) recorded a higher range (319·5–340·7 mg) of pupal weight at 27°C and 85% relative humidity. A considerable decrease in pupal weight was recorded after 20–22 days of pupal formation on different foods. On all foods, male pupa (111·9–153·6 mg) was heavier than female pupa (98·5–127·5 mg). Inspite of highest larval weights on napier grass, pupae of both sexes were lighter in weight in comparison to that of others. However, maximum pupation (38·9%) was recorded on napier followed by sorghum (30·3%), Johnson grass (25·0%) and maize (19·7%). On sugarcane only 3·3% larvae pupated. Under favourable conditions more than 80% pupation was recorded on maize and sorghum (Dhaliwal and Bains 1978; Alam and Khatri 1980). Ratios of males and females at pupal stage were almost equal on different foods.

As is evident from table 3, shortest larval period (28·2 days) was recorded on napier grass followed by sorghum (28·3 days) and Johnson grass (29·3 days) without any significant difference among them whereas, it was longest (49·8 days) on sugarcane at prevailing temperature (18·6 ± 3·2°C) and relative humidity (54 ± 5%). During colder months (October hatching) larval stage had been observed to go upto 100 days on maize (Bindra and Singh 1973), but under favourable conditions it was 15·11–15·18 days on Johnson grass and maize (Kushwaha and Bhardwaj 1977), 19·3–20·75 days on sorghum and maize (Alam and Khatri 1980) and 14–22 days on

Table 2. Effect of feeding on some grasses on pupal parameters of *M. separata* under laboratory conditions.

Test plant	Size of pupa (mm) (2–4 days old)	Pupal weight (mg)			Pupation (%)	Sex ratio	Larval growth index
		2–4 days old	20–22 days old				
			Female	Male		Male : Female	
Napier grass	16·49	236·2	98·5	111·9	38·9	1 : 0·82	1·38
Sorghum	15·66	249·5	127·5	153·6	30·3	1 : 1·10	1·07
Johnson grass	15·24	193·7	112·4	127·1	25·0	1 : 1·10	0·85
Maize	16·91	229·4	111·3	157·0	19·7	1 : 0·85	0·61
Sugarcane	15·00	184·9	101·2	131·1	3·3	1 : 1·00	0·06
Average	16·07	218·7	110·2	136·1	23·5	1 : 0·97	
SEM ±	0·28	13·7	12·1	13·8	3·7		
C.D. at 5%	0·68	31·5	NS	31·8	8·4		

Mean temperature = 18·2 ± 3·9°C. Mean relative humidity = 63 ± 4%.

Table 3. Effect of feeding on some grasses on post embryonic development of armyworm *M. separata* under laboratory conditions (15th November 1984 to 7th March 1985).

Test plant	Developmental durations (days)*				Adult longevity (days)	Moth emergence (%)	Crippled moths (%)		Total growth index
	Larval	Pre-pupal	Pupal	Total			Male	Female	
Napier grass	28·2	2·8	43·8	74·8	3·2	3·4	100·0	—	0·04
Sorghum	28·3	3·5	39·3	71·2	3·3	16·9	28·7	0·0	0·24
Johnson grass	29·3	4·7	35·8	69·8	2·8	4·4	100·0	—	0·06
Maize	32·2	4·7	38·9	75·8	3·5	16·6	60·0	—	0·22
Sugarcane	49·8	10·3	49·7	109·8	3·0	50·0	0·0	0·0	0·45
Average	33·6	5·2	41·5	80·3	3·2	14·5	57·7	0·0	—
SEM ±	1·1	0·4	2·1	2·8	0·4	9·5			
C.D. at 5%	2·6	0·8	4·9	6·1	NS	21·8			
Mean temperature (°C)	18·6 ± 3·2	16·5 ± 2·7	18·2 ± 3·9		18·2 ± 3·2	16·2 ± 2·0	16·2 ± 2·0	16·0 ± 1·8	16 ± 1·8
Mean relative humidity (%)	55 ± 5	52 ± 4	63 ± 4		58 ± 5	60 ± 4	60 ± 4	66 ± 3	66 ± 3

*Based on 300 larvae.

sugarcane (Avasthy and Chaudhary 1965). The larval durations in the present studies were 10–12 days longer than that reported by various workers. This may be because of low temperature and humidity. Pre-pupal period ranged from 2·8–10·3 days. Bindra and Singh (1973) also reported prolonged pre-pupal period of 11 days in January on maize due to low temperature. Pupal period (35·8 days) was shortest on Johnson grass and longest (49·7 days) on sugarcane. Due to low temperature conditions pupal stage of 36–48 days was also reported by Bindra and Singh (1973) and Dhaliwal and Bains (1978). However, under favourable temperature conditions, it ranged from 8–13 days only on different crops (Avasthy and Chaudhary 1965; Bindra and Singh 1973; Kushwaha and Bhardwaj 1977; Singh and Rai 1977; Alam and Khatri 1980).

Contrary to larval survival, moth emergence was maximum (50·0%) on sugarcane and minimum (3·4%) on napier grass. Higher pupal mortality on napier grass was probably because of higher rate of loss of weight in pupa at 18·2 ± 3·9°C and 63 ± 4% relative humidity in comparison to sugarcane and sorghum. In addition to poor moth emergence, male adults showed crippling of wings in napier and Johnson grasses. However, in sugarcane not a single crippled moth was recorded. It was probably due to prolonged larval and pupal durations which resulted in moth emergence in the end of February when the laboratory temperature became more suitable for moth emergence. On the other hand due to short larval period on some grasses, the pupae were exposed to low temperature for a longer time, which was unfavourable for the emergence of moths. Female pupae were observed to be affected more adversely by low temperature (16·0 ± 1·8°C) and relative humidity (66·0 ± 3%) as not even a single female emerged on napier, Johnson grass and maize. As a result total growth index was poor on these foods. Crippled moths were found to be short lived without participating in reproductive process. Females emerged on sugarcane and sorghum laid 400–900 eggs when provided with 10% sugar solution. Hence sugarcane and sorghum were found better food plants of this pest for survival during winter.

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Food utilization and surfacing activity of *Channa striatus* fry in relation to quality of food

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Abstract. Effects of food items (mosquito larva, pupa, chironomous larva and chopped pieces of the fish *Lepidocephalichthyes thermalis*) on food utilization and surfacing activity of *Channa striatus* fry were studied. Test individuals fed on live food organisms surfaced 94–117% more frequently, consumed 23–119% more rations/day and, on an average, converted 33% more than those fed on pieces of *Lepidocephalichthyes thermalis*. Regardless of the quality of food, *Channa striatus* surfaced about 60 times/h before each meal and the frequency increased to 130 times/h 3–6 h after the meal was offered and returned to the prefeeding level 18–21 h after the meal.

Keywords. *Channa striatus* fry; quality of food; food utilization; surfacing activity.

1. Introduction

Culture of air-breathing fishes is gaining much importance in tropical fisheries due to high demand for these fishes. Though sturdy as adults, the fry of the obligatory air-breathing fish *Channa striatus* have low survival rate and hence, pose considerable problems to fish culturists. Non-availability of suitable food organisms and lack of favourable conditions during the development of the air-breathing organ are the two major reasons for high mortality rate of fry of air-breathing fishes (Pandian 1980). As an attempt to select suitable food, fry of *C. striatus* were offered with 4 live food organisms, chopped pieces of the fish *Lepidocephalichthyes thermalis* and the pattern of food utilization and surfacing activity was studied.

2. Materials and methods

Active and well acclimated *C. striatus* fry weighing 308·5 ± 25·91 mg were randomly chosen and divided into 5 series. Four replicates were maintained for each series. The first 3 series were fed on mosquito larva or pupa, the IV series on chironomous larva and the V series on chopped pieces of the fish *L. thermalis*. Chironomous larvae were collected from the field every day, starved for 24 h and then served as food. *Culex* mosquito egg raft was collected from the field regularly, hatched and reared in the laboratory up to 4th instar or pupa (Ponniah and Pandian 1977). Third and fourth instar larvae of *Anopheles* were collected directly from the field and offered as food. *L. thermalis* was maintained separately in aquaria and were starved for 24 h before being offered as food. Food was offered twice a day at 0900 and 1900 h for a period of 1 h each; unconsumed food was collected by a pipette without disturbing the test animals.

Experiments were conducted in cylindrical aquaria (5 cm dia) containing constant depth of water (15 cm). Faecal matter was collected by filtering the aquarium water daily before changing water. Sacrifice method was followed to estimate the initial water and energy contents of the test fish. Energy content was estimated in a Parr 1412 semi-micro bomb calorimeter. The scheme of energy budget followed in the present study is that of IBP formula (Petrusewicz and Macfadyen 1970) represented as $C=P+R+U+F$ where C is the energy consumed, P the growth, R the energy lost as heat due to metabolism, F the faeces and U the nitrogenous waste. Estimation of various energy components has been detailed elsewhere (Sampath and Pandian 1980).

Number of surfacing by each test individual was observed everyday for a known period of time (15 min) during the following timings: 3, 6, 9, 12, 15, 18, 21 and 24 h after feeding. The distance travelled per individual was estimated by multiplying the mean number of visits per unit observations with twice the depth of water. Experiments were conducted for a period of 21 days at a room temperature of $28\pm2^{\circ}C$.

3. Results

Of the 5 test series, the one fed on *Anopheles* larva exhibited the highest rates of feeding, absorption, metabolism and conversion (table 1); these rates were statistically significant compared to the corresponding rates of fry fed on other test series. The series fed on pieces of *L. thermalis* exhibited the lowest rates of feeding, absorption and metabolism. Test individuals fed on live food organisms surfaced 94–117% more frequently, consumed 23–119% more ration and, on an average, converted 33% faster than those fed on pieces of *L. thermalis*. Contrary to this, absorption efficiency did not vary much among the test series and averaged to 90·4%.

Table 1. Effects of different food materials on food utilization and surfacing activity in *C. striatus*.

Parameter	*Anopheles* larva	*Culex* pupa	*Culex* larva	*Chironomous* larva	Pieces of *L. thermalis*
Feeding rate	1895 ± 134·9	1382 ± 176·7	1069 ± 97·7	1122 ± 58·7	868 ± 172·9
Absorption rate	1706 ± 141·1	1227 ± 165·4	963 ± 105·8	1054 ± 59·2	817 ± 213·5
Conversion rate	666 ± 75·3	444·1 ± 64·5	291 ± 35·9	288 ± 36·0	318 ± 68·9
Metabolic rate	936 ± 59·9	703 ± 108·7	605 ± 69·3	690 ± 4·4	432 ± 128·5
Absorption efficiency	89·5 ± 1·30	88·7 ± 0·75	90·1 ± 1·05	94·0 ± 0·41	90·0 ± 2·75
Conversion efficiency(K_2)	38·9 ± 1·27	36·4 ± 2·70	30·5 ± 6·85	27·3 ± 1·84	37·2 ± 5·10
Surfacing frequency/day	2196 ± 315·0	2052 ± 286·2	1962 ± 244·8	2106 ± 201·6	1010 ± 114·0
Hanging duration	3·0 ± 0·25	3·3 ± 0·26	3·0 ± 0·13	2·9 ± 0·38	—
Distance covered	539 ± 74·90	508 ± 63·50	577 ± 82·60	549 ± 51·75	222 ± 25·10

Each value represents the average (± SD) performance of 4 individuals at $28\pm2^{\circ}C$. Rates are expressed in J/g live fish/day, efficiencies in percentage; hanging duration and distance covered are expressed as h/day and m/day, respectively.

Conversion efficiency ranged from 27·3% (series fed on Chironomous larva) to 38·9% (series fed on *Anopheles* larva) and averaged to 34·1%.

The series fed on live food organisms surfaced twice (average: 2079 times/day) as frequently as the series fed on *L. thermalis* pieces (1010 times/day); correspondingly, the distance covered to surface increased by nearly 2·5 times. After undertaking a few surfacings, *C. striatus* resorts to a short resting period in water surface called hanging. In the present experiment, the duration of hanging did not change in any of the test series and remained around 3 h/day (table 1).

Hourly fluctuation in surfacing frequency following a meal was observed in the test series fed on live organisms. This study was conducted during the last 3 days of the experiment over a 24 h cycle. The test individuals surfaced about 60 times/h before feeding and the frequency increased to about 130 times/h, 3–6 h after the meal in the series fed on *Anopheles* larva and decreased thereafter to the prefeeding level 21 h after each meal (figure 1). Vivekanandan *et al* (1977) have reported similar trend in the surfacing frequency following meal in another obligatory air-breathing fish *Anabas scandens*.

4. Discussion

The test series which were offered 4 different live food organisms exhibited statistically significant difference in food utilization parameters, viz rates of feeding, absorption, conversion and metabolism as compared to those fed on pieces of *L. thermalis*.

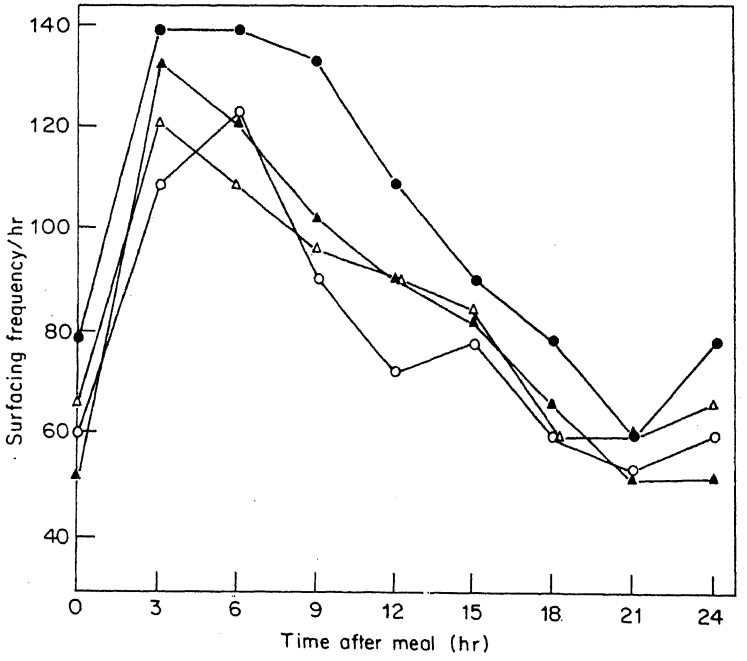

Figure 1. Hourly fluctuation in the surfacing frequency/h as a function of time after meal in *C. striatus* fed on different live food organisms (●), *Anopheles* larva; (△), *Culex* pupa; (○), *Culex* larva; (▲), Chironomous larva.

Table 2. Water content and caloric value of different food materials and experimental fish.

	Food		Fish	
Food	Water content (%)	Caloric value (kJ/g dry wt)	Water content (%)	Caloric value (kJ/g dry wt)
Anopheles larva	83·4 ± 8·20	21·7 ± 0·51	76·7 ± 0·69	19·7 ± 0·57
Culex pupa	88·7 ± 7·50	21·0 ± 0·90	77·5 ± 1·01	19·0 ± 0·85
Culex larva	88·0 ± 7·90	22·1 ± 0·77	77·0 ± 0·87	19·4 ± 0·61
Chironomous larva	84·6 ± 7·50	23·1 ± 0·90	76·7 ± 0·17	19·7 ± 0·54
L. thermalis pieces	80·0 ± 6·70	19·5 ± 0·28	79·2 ± 0·69	19·5 ± 0·57

This result provides clues on the possible strategies adapted by *C. striatus* when exposed to different food items: (i) when exposed to low energy food *L. thermalis* (19·5 kJ/g dry wt; table 2), *C. striatus* reduces the feeding rate and compensates by decreasing the surfacing frequency and alloting less energy for swimming activity and metabolic rate, (ii) the high feeding rate in the series fed on *Anopheles* larva might have resulted from a behavioural synchronisation of the properties of the predator and prey. *Anopheles* larva stays at the water surface most of the time and, while surfacing, *C. striatus* encounters *Anopheles* larva more frequently than other food organisms. This synchronisation might have resulted not only in higher feeding and conversion rates but also in the least allocation of ingested energy towards metabolism, and (iii) there is no evidence of changes in feeding, absorption and conversion rates and surfacing activity among the series receiving live food organisms in relation to the minor variation in the energy content. However, the series receiving the lowest food energy (*L. thermalis*) consumed the least ration. As the conversion rate of the series fed on *Anopheles* larva (666 J/g/day) was substantially faster than the rates exhibited by the other series (288–444 J/g/day), it may be advantageous to feed *C. striatus* fry on live food organisms which remain at the water surface.

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On some plant galls from the Fiji Islands

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Abstract. This paper describes 34 new galls and records one widely distributed gall, collected by the first author in the course of a two-month visit to the Fiji Islands. The midge galls include those on *Flacourtia* sp., *Calophyllum* sp., *Heritiera ornithocephala*, *Canarium vitiense*, *Syzygium rubescens*, *Astronidium* sp., *Plerandra vitiense*, *Tapeinosperma* sp., *Pagiantha thurstonii*, *Cyrtandra* sp., *Glochidion ramniflorum*, *Gironniera celtidifolia*, *Gnetum gnemon*, etc. The Eriophyid galls are on *Hibiscus schizopetalus*, *Hibiscus tiliaceus*, *Brucea javanica*, *Vitis vitifolia*, *Pometia pinnata*, *Ipomaea gracilis*, *Ipomaea* sp., *Macaranga magna* and *Nephrolepis biserrata*. The Psyllid galls occur on *Canarium* sp., *Haplolobus floribundus*, *Maesa tabacifolia*, *Myristica micrantha*, *Cryptocarya* sp. The dominance of leaf galls and midge galls is considered as strong evidence of the relatively recent origin of the flora of these islands.

Keywords. Zoocecidea; Fiji.

1. Introduction

This paper deals with the plant galls, collected by the first author, during a two-month visit in 1978 to the Fiji Islands. The collections were made in the course of a series of field trips in the Viti Levu and in the Taveuni Islands. Of the 35 galls described here, 34 are new to science. Except one gall on the shoot axis, all the others occur on leaf. The majority of the galls are caused by Cecidomyiidae, 9 galls are caused by Eriophyidae and only 6 by Psyllidae. Except for one gall each on a Pteridophyte and Gymnosperm, all the other galls occur on Dicots.

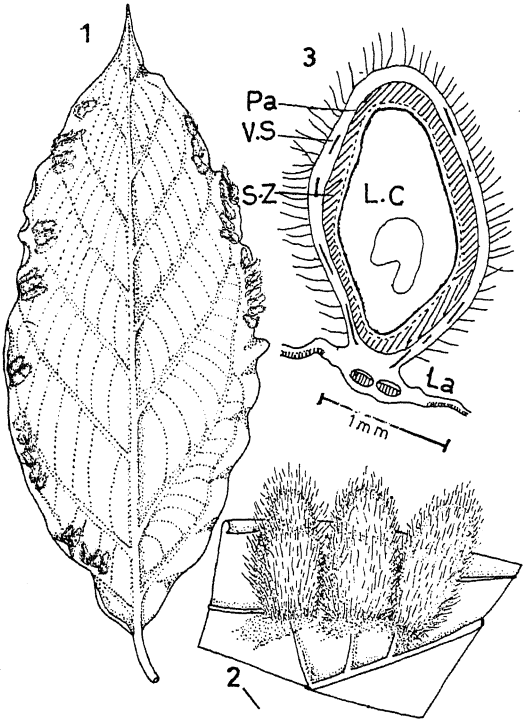
The Fiji Islands in southwest Pacific Ocean, about 2600 km to the northeast of Australia, comprise nearly 500 islands, of which 97 are inhabited, mostly in the coastal areas and in the river valleys. Of volcanic origin, the islands are generally rugged and mostly below 1250 m above mean sea-level and are characterized by mild climate. In the larger islands there is a wet zone on the windward coast and a dry zone on the leeward coast, with characteristic vegetation. The flora is remarkable for the presence, besides many introduced species, of outliers of the Oriental elements, which have bypassed Australia over Borneo-Sulawesi and New Guinea group of islands to reach their extreme eastern limits. The dominance of leaf galls and of the galls by Cecidomyiidae over Eriophyid galls and the high endemism of the galls are strong evidence of a relatively recent origin of flora of these islands.

2. Family Flacourtiaceae

Flacourtia sp. (figures 1–3)

Gall No. 840 Cecidomyiidae

Foliar gall; hypophyllous, erect, flask-shaped, hollow, densely bristled, closed,



Figures 1–3. *Flacourtia* sp. **1.** A leaf showing the distribution of galls. **2.** Three galls enlarged. **3.** L.S. of the gall. (La, Lamina; LC, larval chamber; Pa, parenchyma; SZ, sclerenchyma zone; VS, vascular strands).

covering-growth sessile pouch-gall, crowded together in two submarginal rows of the leaf; about 4 mm long and 2 mm thick.

The gall appears like a miniature sea-urchin due to the occurrence of dense, stiff, straight unicellular unbranched and heavily thick-walled acute bristles on the surface; gall cavity axial, elongate, heavily sclerotized. A zone of collapsed cells surrounds the gall cavity, followed by 4–5 layers of brachysclereids, sclereids radially elongated, progressively larger, with well developed ramiform canals in walls and wide cell lumen; external to the sclerotic zone are compact, vertically elongated and thin walled parenchyma cells, delimited by a distinct epidermis, with elongated cylindrical cells; trichomes deep-seated in the outer layers of the parenchyma zone.

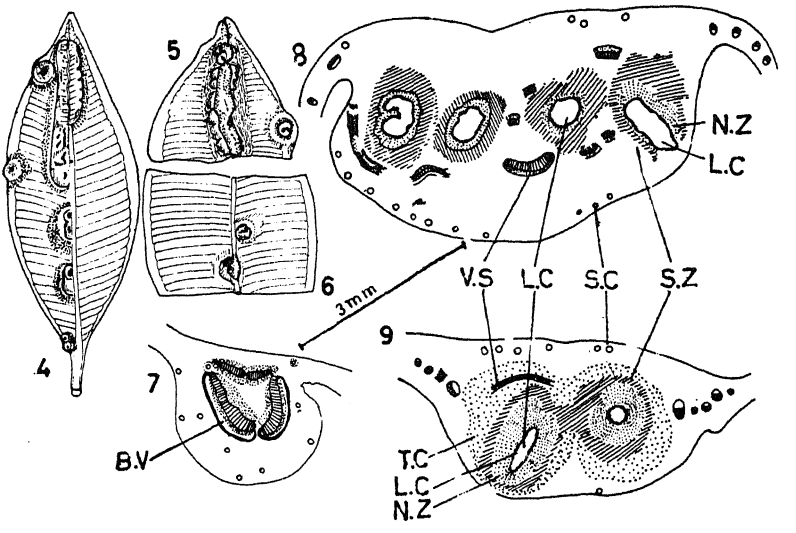
Locality: Taveuni: Soqulu Estate: Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

3. Family Guttiferae

Calophyllum sp. (figures 4–9)

Gall No. 841 Cecidomyiidae

Foliar gall; hypophyllous unilateral or bilateral fleshy, lobed, short or also extensive, glabrous, indehiscent, multilocular swellings of the midrib, with the lamina depressed



Figures 4–9. *Calophyllum* sp. **4.** Adaxial view of a gall bearing leaf. **5 and 6.** Adaxial view of the galls on two leaf segments. **7.** T.S. of midrib. **8.** T.S. of the midrib gall. **9.** T.S. of the laminar gall. (BV, Bicollateral bundle; LC, larval chamber; NZ, nutritive zone; SC, secretory canal; SZ, sclerotic zone; TC, tanniniferous cells; VS, vascular strands).

on either side of the galled vein; about 0·5–0·7 cm thick; lateral fleshy outgrowths of gall are 0·3 cm thick; also on leaf margin as circular hypophyllous discs, with a central depression, about 1 cm in diameter; minute exit holes on the abaxial side of gall.

When the gall develops unilaterally on the midrib, the vascular tissues of the vein remain unaffected. The parenchyma of cortex of the vein and lamina contribute to the formation of the gall, and undergo extensive hyperplasia and hypertrophy, resulting in the compact homogenous mass of gall cells; when the gall develops bilaterally, the gall is larger than when unilateral and the vascular tissues become broken up into distorted strands by proliferation of medullary and cortical cells of the midrib.

Larval cavities in a horizontal row both in the unilateral and bilateral galls, irregular, surrounded by well developed nutritive and sclerotic zones; cells immediately lining the larval cavities collapsed; cells inner to the band of crushed cells intact and in radial rows, with dark granular contents; sclerotic zone consists of compact brachysclereids, with large lumens, filled with tannin.

The galls on leaf margin are basically similar to those of veins, hypophyllous, with two or more larval cavities, surrounded by the nutritive and the sclerotic zones, outside of which is a broad zone of tanniniferous cells, absent in the vein galls; mesophyll tissues have lost their identity, and have proliferated in the vertical plane at right angles to the surface, resulting in homogenous mass of cell layers.

Locality: Taveuni: Soqulu Estate: Coll. M S Mani and M K Kamath, 19 December 1978.

4. Family Malvaceae

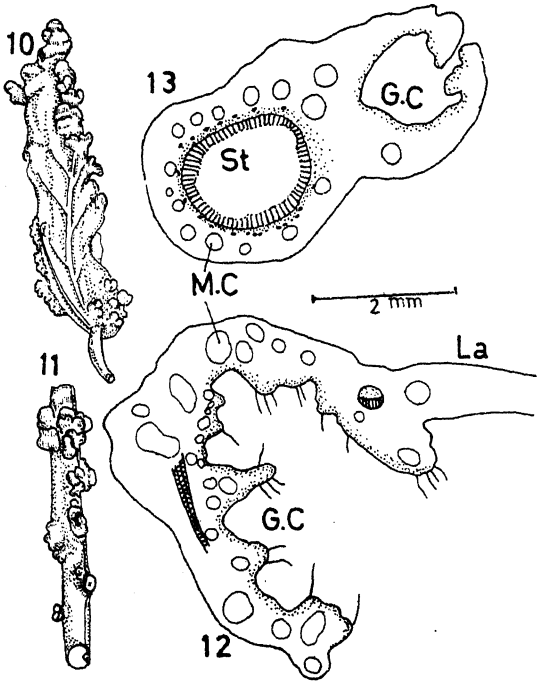
4.1 *Hibiscus schizopetalus* Hook. f. (figures 10–13)

Gall No. 873 *Eriophyes* sp.

Gall occurs on leaf, petiole and stem. The foliar gall irregular, epiphyllous fleshly agglomerate mass of pouch, rather clustered towards the major veins, with a wide opening on the opposite side; galls on stem and petiole irregular covering growths with wide apical ostiole; foliar gall 2 mm high and 3 mm in diameter and the petiole and stem galls slightly smaller; both yellowish-green, soft, glabrous.

The foliar gall epiphyllous, shallow, fleshy pouch, with wide hypophyllous opening and irregular fleshy emergences from the inner surface of the cavity; stellate hairs persist unmodified on the fleshy emergences in the cavity; mucilage cavities larger and more numerous than in normal leaf; epidermis bordering the gall cavity with hypertrophied cells, enclosing prominent nuclei and dense cytoplasm; gall parenchyma consists of compact, thin-walled, irregular parenchymatous cells.

The petiole and stem galls essentially similar, arise as covering growths from proliferation of cortical cells, without affecting the vascular tissues; considerably hypertrophied and homogenous; cells around the gall cavity possess dense cytoplasm



Figures 10–13. *H. schizopetalous* Hook. **10.** A leaf with galls. **11.** Stem with galls. **12.** T.S. of foliar gall. **13.** T.S. of stem gall. (GC, Gall chamber; La, lamina; MC, mucilage canals; St, stem).

and giant nuclei; cavity smooth and wide; ostiole narrow; no hairs on the lining layer of the cavity; mucilage cavities of the gall larger than those in the normal portion.

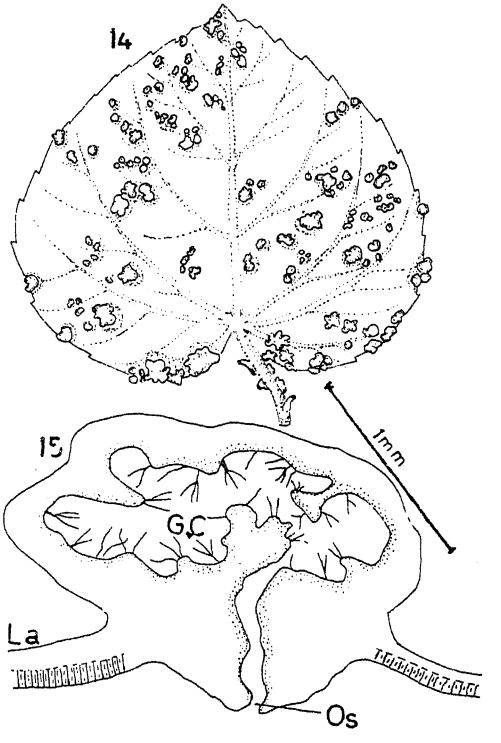
Locality: Taveuni: Waiyevo: Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.

4.2 *Hibiscus tiliaceus* Linn. (*figures* 14 *and* 15)

Gall No. 832 *Eriophyes hibiscitileus* Nalepa

Leaf gall; ephiphyllous or hypophyllous, rugose, pustuloid, pale yellow, unilocular pouch-gall; gall cavity large, filled with emergences from the wall of the gall; ostiole minute on the minute hypophyllous covering growth; on stem and petiole the gall arises as small covering growth, with the ostiole at the tip of the gall; size 8–14 mm in diameter.

Proliferation of the ground tissues gives rise to homogenous compact layers of cells; several fleshy, lobed emergences grow from the wall of the gall cavity; outer surface of gall densely pubescent like on normal leaf; surface layer of the gall cavity has the same type of stellate hairs as the normal leaf; gall trichomes have a distinct pedicel, from the top of which the arms of the trichome spread horizontally; larger than those of the leaf; ostiole is narrow and straight; galls on stem and petiole flask-



Figures 14–15. *H. tiliaceus* Linn. **14.** A leaf with galls. **15.** T.S. of leaf through a gall. (GC, Gall chamber with fleshy outgrowths and trichomes; La, lamina; Os, ostiole).

shaped with narrow, outwardly bent neck; basically similar to the leaf gall except that they are covering-outgrowths formed by the proliferation of the outer cortical parenchyma.

Locality: Viti Levu: Deuba beach side: Coll. M S Mani, Sundaresan and M K Kamath, 12 December 1978.

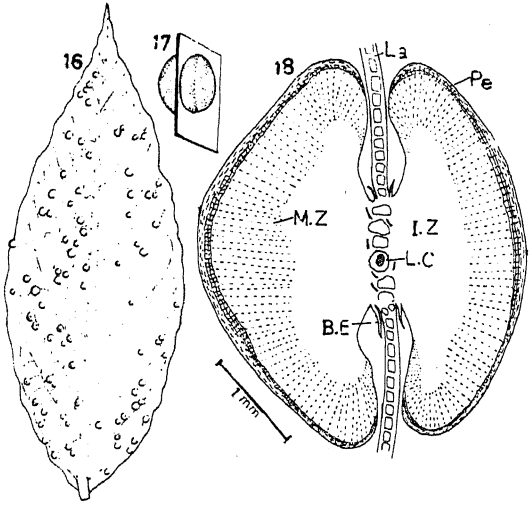
4.2a *Remark*: This gall is reported by Mani (1973) as having wide distribution in India, Hongkong, Celebes, Malaya, Bismarck Archipelago, Samoa and Java (Houard C; 1922, 1923, Les Zoocecidies des Plantes d'Afrique, d'Asie et d'Oceanic, 1: 1–498; 2: 499–1058; Houard, C; 1933, Les Zoocecidies des Plantes de l'Amerique du sud et l'Amerique Centrale, pp. 1–519; Mani M S; 1948, Zoocecidia and Cecidozoa from India, J. R. Asiatic Soc. Bengal, (Sci.) 14 (2): 4348; Mani M S; 1973, Plant galls of India, Macmillan India, pp. 1–354; Drs Van Leeuwen-Rajnvaan W M; 1926, The Zoocecidia of the Netherlands, East-Indies; Rübsaamen E H; Beiträge zur Kenntnis aussereuropäischer Zoocecidien, Marcellia 4: 65–138; 6: 110–173; 7: 15–79).

5. Family Sterculiaceae

Heritiera ornithocephala Kosterm. (figures 16–18)

Gall No. 860 Cecidomyiidae

Foliar twin-gall; short hour-glass shaped gall of two semi-circular, subsessile halves, one on each side of blade; the halves joined together at the blade by a small neck-like interconnecting portion; the two halves equal or rarely one may be smaller; smooth,



Figures 16–18. *H. ornithocephala* Kosterm. **16.** A leaf with galls. **17.** A gall enlarged. **18.** Vertical section of a gall. (B E, Broken epidermis; I Z, inner zone of parenchyma; La, lamina; L C, larval chamber; M Z, middle zone of parenchyma; Pe, periderm).

dark brown, nut-like and indehiscent; about 3·5 mm in diameter and 3 mm in height.

This is a remarkable eruption gall, of two structurally identical, dome-shaped bodies, above and below the blade, exactly opposite to each other; two plano-convex halves originate from a common point on the lamina and have a short, stout, interconnecting neck; each has thus the appearance of an umbrella-shaped body. The larval cavity small, circular, in the middle of the neck, equidistant from the two halves, with a single minute larva. The parenchyma cells divide periclinally and later elongate vertically; consequently erupting out by rupturing the leaf epidermis on both surfaces, the broken epidermis pushed aside as a circular membrane around the neck of the gall on both the surfaces of the blade; each half of the gall consisting of 3 zones: the outermost of a narrow band of radial series of laterally stretched cells, which resemble the periderm tissue; the outermost layers of the zone are collapsed and remain as a dark line; the next inner zone of large, vertically elongated tanniniferous cells with dense aggregation of starch grains; third zone extends down into the neck, and consists of smaller, compact irregular cells; these cells also contain starch grains, but no tannin; in the region of the leaf where the galls are attached, the air-chambers are larger than those of the normal region and are also irregular in outline. The tracheary elements are scattered in horizontal plane in this region. The larval chamber is surrounded by smaller densely cytoplasmic cells.

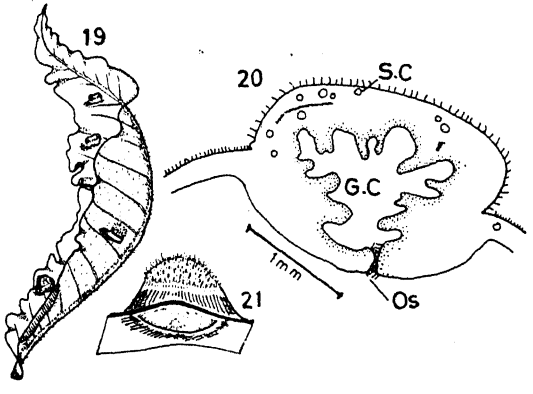
Locality: Taveuni: Soqulu Estate: Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

6. Family Simaroubaceae

Brucea javanica (Linn.) Merrill (figures 19–21)

Gall No. 838 *Eriophyes* sp.

Foliar gall; epiphyllous, sometimes hypophyllous, hemispherical, sessile, hairy, multilocular, brownish pouch gall; sparsely scattered, about 3–4 mm in height and 5 mm in width.



Figures 19–21. *B. javanica* (Linn.) Merrill. **19.** A leaf with gall. **20.** Vertical section of a gall. **21.** A gall enlarged. (G.C, Gall chamber; Os, ostiole; S.C, secretory cavity).

The gall consists of an epiphyllous. hemispherical pouch and equally large projecting hypophyllous covering growth, with fleshy emergences projecting from the inner surface of the gall cavity, dividing the cavity into a multilocular chamber; straight, thick-walled hairs line the ostiole and the outer surface of the gall; similar to, but denser than those on the normal lamina; gall tissue consists of much hypertrophied, compact, polygonal cells, without much differentiation into the usual components of the mesophyll tissues; tanniniferous cells abundant in the gall tissue; cells lining gall cavity cytologically distinct from the remaining layers, radially elongated, with prominent nuclei and dense cytoplasm.

Locality: Taveuni: Soqulu Estate: Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

7. Family Burseraceae

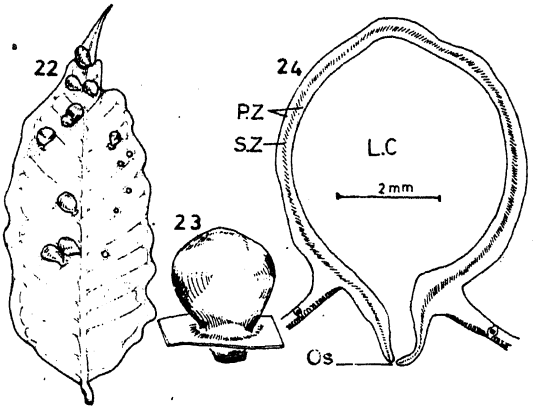
7.1 *Canarium vitiense A Gray (figures 22–24)*

Gall No. 853 Cecidomyiidae (?)

Hypophyllous, globose or elliptical, large glabrous, thin-walled, indehiscent, reddish-brown, sessile or shortly necked pouch gall, upto 10 galls per leaf; with a small conical epiphyllous, ostiolate, covering growth, 1 mm long, gall about 7 mm high and 5 mm wide.

The gall cavity large, with smooth inner and outer surfaces; there are 4–5 layers of laterally stretched cells in the inner part of the gall, followed by 2–3 layers of sclerenchyma zone. The outer part of the gall consists of a few layers of large thin-walled cells, which are collapsed in the periphery. The sclerenchyma cells are large with wide lumen.

Locality: Viti Levu: Colo-i-Suva: Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1978.



Figures 22–24. *Canarium vitiense* A. Gray. **22.** A leaf with galls. **23.** A single gall enlarged. **24.** Vertical section of a gall. (L.C, Larval chamber; Os, ostiole; PZ, parenchyma zone; SZ, sclerotic zone).

7.2 *Canarium sp. (figures* 25–27)

Gall No. 862 Psyllidae

Epiphyllous, globose or hemispherical solitary or more commonly agglomerate masses, mostly in the vicinity of the veins, with minute hypophyllous ostiole, and a mature hypophyllous covering growth about 3 mm high and 2–3 mm thick; larval cavity wide and shallow. In the young gall, the larval cavity is surrounded by radial cells from a meristematic zone; outer to this meristematic zone are compact parenchyma irregular cells with undulate walls. In the mature galls, the cells bordering the larval cavity and those at the periphery of the dome are collapsed to form a dense dark layer. The cells in between the disintegrated zones are compact, thin-walled and stretched in the plane parallel to the cavity.

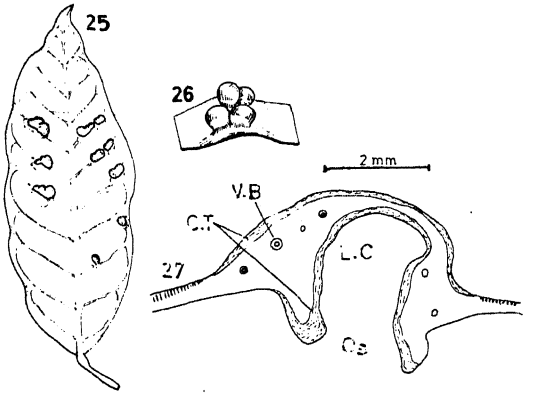
Locality: Taveuni: Soqulu Estate: Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

7.3 *Haplolobus floribundus (Schum) H J Lam. (figures* 28–31)

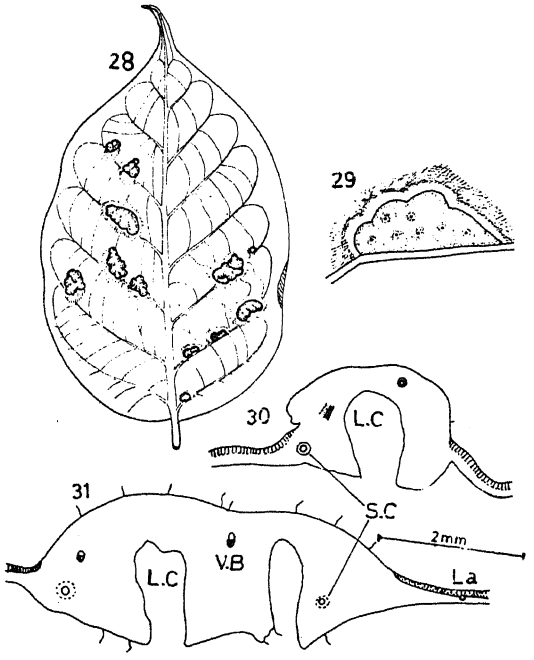
Gall No. 844 Psyllidae (?)

Epiphyllous, slightly raised, irregular, yellowish-green pouches, with circular, wide-open hypophyllous ostiole on hypophyllous short covering growth; solitary or irregular agglomerate mass, especially near the lateral veins. The solitary gall measures 1 mm in diameter and the agglomerate mass measures 1 cm.

Ostiole and the larval cavities are wide; the lamina gradually merges with the gall and the different tissues of the former lose their identity in the gall due to extensive hypertrophy and hyperplasia. In the transition from the normal lamina into the gall, the number of cell layers remain unaltered, but the cells are greatly hypertrophied and their differentiation into the spongy and palisade tissues are suppressed. In the



Figures 25–27. *Canarium* sp. **25.** A leaf with galls. **26.** A group of galls enlarged. **27.** Vertical section of a gall. (CT, Crushed tissues; LC, Larval chamber; Os, ostiole; VB, vascular bundle).



Figures 28–31. *H. floribundus* (Schum.) H J Lam. **28.** Adaxial view of a leaf with galls. **29.** Adaxial view of the gall with ostioles. **30 and 31.** Vertical sections of a solitary and compound galls respectively. (La, Lamina; L C, larval chamber; S C, secretory canal; V B, vascular bundle).

gall proper, the cells have undergone repeated transverse and vertical divisions and are stretched in vertical plane, without differentiating into the usual ground tissues. The cells that would have normally differentiated as palisade and spongy parenchyma have contributed equally to the growth of the gall. Large, circular, schizogenous cavities found in the gall are smaller in the normal lamina. In the roof of the gall cavity, the cells have divided transversely and stretched laterally to form compact rectangular cells. This region of the gall is slightly thicker than the normal lamina. Here also the differentiation of the ground tissues is suppressed. Neither sclerotic nor special nutritive zones are found in the gall.

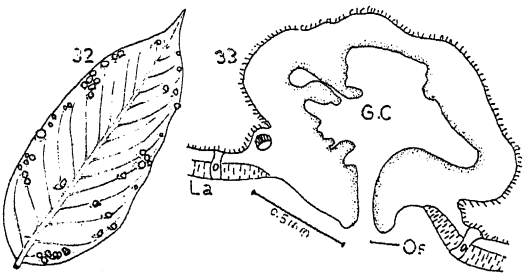
Locality: Taveuni Queleni; Coll. M S Mani, Sundaresan and M K Kamath, 20 December 1978.

8. Family Vitaceae

Vitis vitifolia Lamarck (figures 32 and 33)

Gall No. 870 *Eriophyes* sp.

Hypophyllous, minute, dot-like, scattered, densely hairy, pouch gall, with epiphyllous ostiole, about 1–2 mm high and 1 mm wide.



Figures 32 and 33. *Vitis vitifolia* Lamarck. **32.** Adaxial view of a leaf with galls. **33.** Vertical section of a gall (GC, Gall chamber; La, lamina; Os, ostiole).

The gall consists of a hemispherical hypophyllous pouch, with dense trichomes on the surface similar to those of the normal leaf; ostiole at the tip of a minute epiphyllous conical covering growth, the inner surface of the gall with short, irregular, solid, stout tuberculate outgrowths, no hairs on the lining layer of the gall cavity. The gall consists of about 12 layers of compact, more or less homogenous polygonal cells. The cells of the lining layer of the gall cavity are distinct from the remaining cells in being radially elongated with dense protoplasmic contents.

Locality: Taveuni: Vurevure; Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.

9. Family Sapindaceae

Pometia pinnata J R and G Forst (figures 34 and 35)

Gall No. 835 *Eriophyes* sp.

Epiphyllous, blister-like, rugulose, rusty-brown, irregular swellings of the lamina about 2 cm large; gall cavity shallow and wide, clothed with dense brownish erineum; gall cells exhibit limited hyperplasia followed by hypertrophy, parenchyma compact, without intercellular air spaces; anticlinal divisions of both dermal and parenchyma and subsequent enlargement of the derivatives cause the invagination of the lamina into a shallow pouch; dense erineum lining the gall cavity consists of unicellular, unbranched hairs, longer and broader than those of the normal leaf.

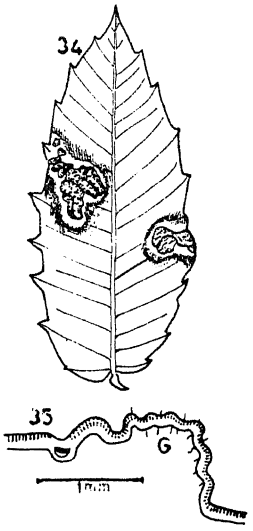
Locality: Viti Levu: Deuba; Coll. M S Mani, Sundaresan and M K Kamath, 12 December 1978.

10. Family Myrtaceae

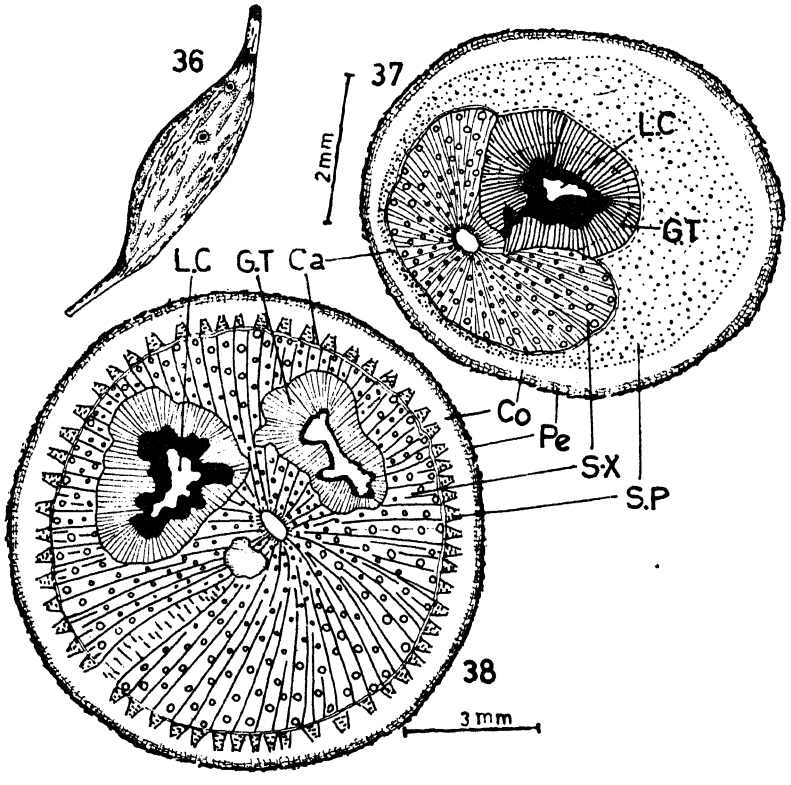
10.1 *Metrosideros sp. (figures* 36–38)

Gall No. 875 Cecidomyiidae midge

Stem gall; extensive fusiform or localised globose hard, smooth, indehiscent swellings of the internodes, with minute exit holes; numerous minute larval cavities as dark,



Figures 34 and 35. *Pometia pinnata* J R and G Forst. **34.** Leaf with galls. **35.** T.S. of leaf passing through the gall (G, Galled portion of the leaf).



Figures 36–38. *Metrosideros* sp. **36.** A galled twig with exit holes. **37.** T.S. of a young gall. **38.** T.S. of a mature gall. (Ca, Cambium; Co, cortex; G T, gall tissue; L C, larval chamber surrounded by dark crushed tissues; Pe, periderm; S P, secondary phloem; S X, secondary xylem).

irregular spots within the secondary xylem cylinder; gall measures 3–5 cm long and 1 cm in diameter.

In a young gall, the larval cavity is external to the vascular cambium and is surrounded by a dark band of collapsed tissue, which is again followed by radiating rows of thin-walled, unlignified tissue. The minute larval cavity and its surrounding cylinder of radiating cells appear as if embedded in the furrow of the vascular cylinder of the stem. The gall tissue cylinder originates by the centripetal activity of a circular meristem. As the girth of the gall tissue increases, it inhibits the activity of a strip of cambium adjacent to it, while the remaining portion functions normally. This causes the furrowing of the vascular cylinder and thrusting of the gall tissue cylinder into the furrow. The normal vascular tissues and the gall tissues grow as coaxial cylinders; however, the activity of the vascular cambium supersedes the growth of the gall tissue and the secondary xylem produced by the cambium grows over and around the gall tissue cylinder, thus engulfing it. At a later stage, the cambium forms a complete cylinder, so that its derivatives push the larval cavity and its surrounding reaction tissues deep into the xylem cylinder. Thus, in a mature gall, there is a broad, complete cylinder of secondary xylem with the central pith, and one or more intraxylary larval cavities, each surrounded by its own reaction tissues.

The cells around the larval cavity resemble the secondary xylem, but are isodiametric and lack the rays, fibers and vessels. These cells and those in the vicinity of the gall tissue are larger in size, thin walled and lack lignification. The vascular elements of the galled stem are shorter, broader and sometimes curiously shaped. Proliferation of the cortical tissue also adds to the growth of the gall.

Locality: Viti Levu: Suva; Coll. M S Mani, Sundaresan and M K Kamath, 12 December 1978.

Syzygium rubescens (A Gray) C Muell.

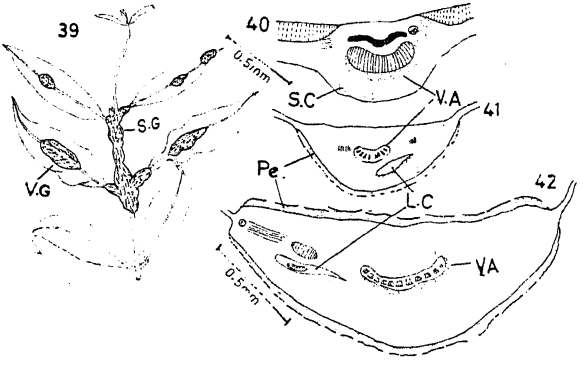
Gall No. 852 Cecidomyiidae midge

The gall occurs on the stem, petiole and veins. On the stem, the gall is a diffuse cylindrical and often a moniliform swelling, extending from node to node; on leaves the gall develops along the entire length of the petiole, as fusiform, much elongated largely hypophyllous swelling on the midrib; both the stem and foliar galls indehiscent, dark brown, rugose and covered with membranous scales; stem gall 7 mm in diameter and the vein gall 4 mm thick and 9 mm broad.

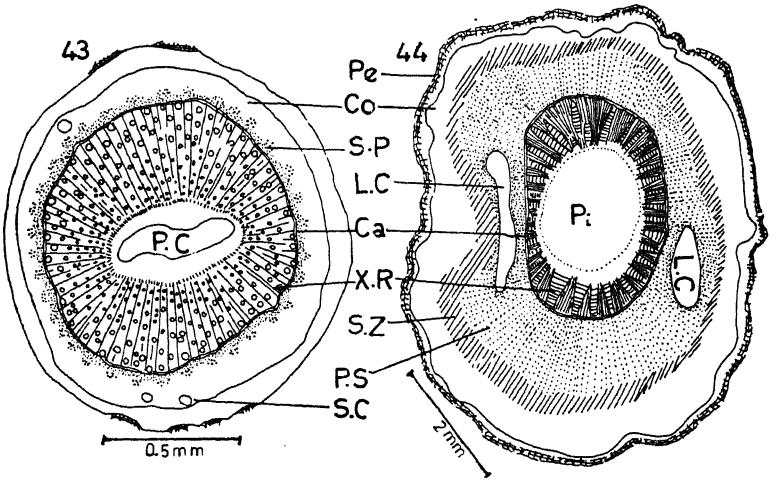
The stem gall is roughly circular in cross section (figures 43–44). There are one or two narrow larval cavities in the region of secondary phloem. The central pith cavity is absent. Due to reduction in the activity of the cambium, the secondary xylem cylinder appears thinner and especially in the vicinity of the larval cavities this tissue is much reduced. The ground tissues have proliferated considerably to contribute to the increase in the thickness of the gall. The secondary xylem cylinder also increased in perimeter concomitant with the increasing girth of the gall and this circumferential increase is by proliferation of the medullary rays which consequently broke the xylem cylinder into narrow radial bands. The ray cells are stretched laterally and the bands of ray are wedge shaped in transection. The secondary phloem also increased and the cortical fibres are better developed forming

a distinct continuous zone. The larval cavity is bound by peculiar type of sclereids, with secondary thickenings only on the inner tangential and part of the radial walls. The periderm of the gall is thicker than that of the normal stem. Tanniniferous cells and druses are abundant in the ground tissues of the gall.

10.2a *Vein gall* (*figures* 39–42): The gall is confined to the midrib. Any part of the midrib such as basal, middle and terminal, may be galled. The gall projects more on the lower side than on the upper side which is flat or even somewhat concave. Along with the vein a part of the lamina on either side is also involved in galling, so that the gall is pronouncedly wide. One or two larval cavities are found on the lower or lateral



Figures 39–42. *S. rubescens* (A Gray) C Muell. **39.** A shoot showing stem and vein galls. **40.** T.S. of mid-vein. **41.** T.S. of gall at the terminal part of the vein. **42.** T.S. of gall at the middle part of the vein. (LC, Larval chamber; Pe, periderm; SC, secretory canal; SG, stem gall; VA, vascular arch; VG, vein gall).



Figures 43 and 44. *S. rubescens* (A Gray) C. Muell. Stem gall; **43.** T.S. of stem. **44.** T.S. of stem gall. (Ca, Cambium; Co, cortex; LC, larval chamber; PC, pith cavity; Pe, periderm; Pi, pith; PS, proliferated secondary xylem; SC, secretory canal; SP, secondary phloem; SZ, sclerotic zone; XR, xylem rays).

part of the vascular arch. The vascular strand expands laterally due to proliferation of the ray cells and consequently the xylem tissue occurs in the form of narrow bands, alternating with the proliferated rays. The ground tissues, especially those bordering the larval cavities, proliferate by periclinal divisions increasing the bulk of the gall. The surface layers of cells also divide periclinally giving rise to a zone of periderm like cells. The periderm like zone may occur on both adaxial and abaxial sides or only on the abaxial side. The sclereids with thickened inner tangential and lateral walls occur a few layers away from the larval cavities. The cells around the larval cavity exhibit features of the nutritive tissues.

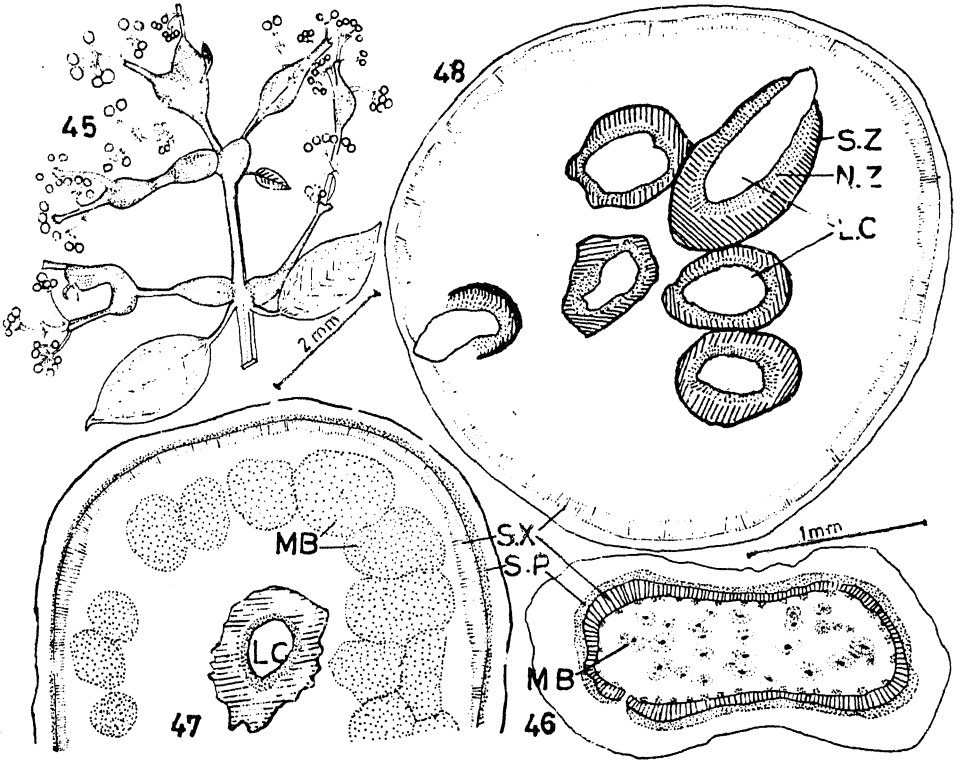
Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

11. Family Melastomaceae

11.1 *Astronidium sp.* (*figures* 45–48)

Gall No. 837 Cecidomyiidae

Large, fusiform or ovoid smooth, hard indehiscent nodal or internodal swellings,



Figures 45–48. *Astronidium* sp. **45.** A flowering shoot with galls on different parts of the axis. **46.** T.S. of stem. **47.** T.S. of a gall with a single central larval chamber. **48.** T.S. of a gall with many larval chambers. (LC, Larval chamber; MB, medullary bundles; NZ, nutritive zone; SP, secondary phloem; SX, secondary xylem; SZ, sclerotic zone).

measuring about 2–3 cm long and 2 cm in diameter on the stem and primary axes of the paniculate inflorescence.

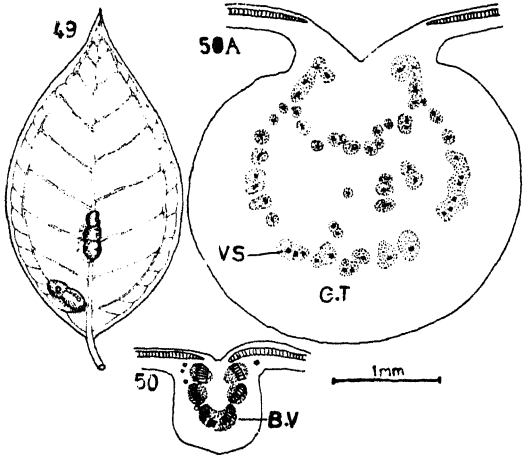
The gall may have either a single axial larval cavity or many scattered cavities, with a single larval cavity having the same tissue systems as in the normal stem, but showing extensive hyperplasia and hypertrophy. The cortical cells of the gall have increased only to a limited extent, while the vascular tissues have increased both in the number of cell layers and in size of the cells. The sieve tubes of the internal phloem are well developed. The pith cells are much hypertrophied and the medullary bundles are dilated. The striking feature of the medullary bundles of the gall is that the differentiation of their xylem elements is completely suppressed and the sieve tubes are well developed. In the gall with many larval cavities the medullary bundles are absent. A functional cambium exists in the gall, and its inner derivatives are found in radial seriations but they do not develop secondary wall. They remain as unlignified parenchymatous tissue. The larval cavities are wider and irregular in outline. Each cavity is surrounded by nutritive cells and sclerotic zones. The nutritive cells have dense granular contents and the sclerotic cells are of brachysclereid type. In certain parts of the gall a distinct periderm is seen which is absent in a normal stem. The effect of the formation of periderm tissue in the gall is to increase circumference of the stem in order to accommodate the increased girth of the gall.

Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

11.2 *Astronidium sp. (figures* 49 *and* 50)

Gall No. 867 Cecidomyiidae midge

Vein gall; spindle-shaped, moniliform, smooth, solid, soft, fleshy, indehiscent swellings of the midrib and lateral veins. The gall projects more on the abaxial side and measures 7 mm long and 3 mm in diameter.



Figures 49–50. *Astronidium* sp. **49.** Abaxial view of the vein gall. **50.** T.S. of vein. **50A.** T.S. of vein gall (BV, Bicollateral vascular bundle; CT. gall tissue; VS, vascular strands - distorted).

The gall has a narrow axial larval cavity, opening to the outside by a minute exit hole in the mature gall. The growth of the gall is due to hypertrophy and hyperplasia of parenchyma, as well as the perivascular and vascular parenchyma. Due to intensive proliferation of the inter and intra-vascular parenchyma, the original vascular bundles are broken into numerous islands, arranged in a wide ring. The procambium of each strand is stimulated to divide periclinally to form radial files of cells. However, the derivatives are not lignified; there are only a few lignified tracheary elements in each strand which are widely spaced. In the medullary portion of the gall are seen a few islands of smaller cells surrounded by larger cells. The epidermis of the gall expands in surface by anticlinal divisions and lateral expansion and keeps pace with the growing size of the gall. The annular sclereids are absent in the gall.

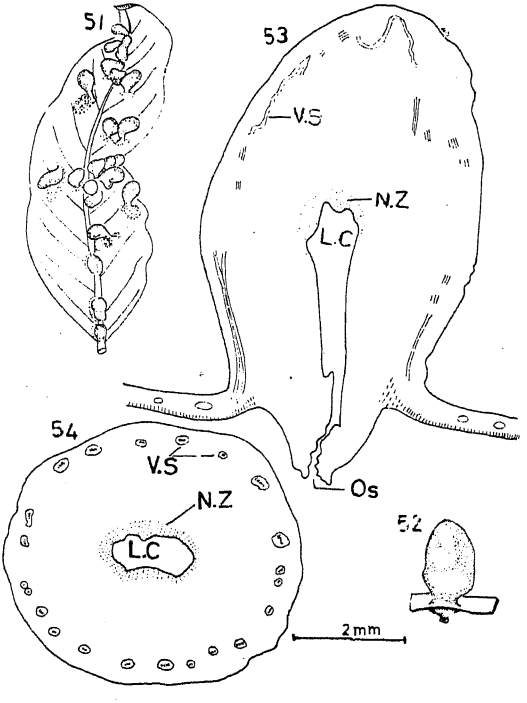
Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

13. Family Araliaceae

Plerandra vitiensis (Seem) Baillon (*figures* 51–54)

Gall No. 849 Cecidomyiidae midge

Hypophyllous, ovoid, shortly pedicellate, fleshy, soft, yellowish-green, with epiphyllous ostiole; either solitary or crowded, erect or lopsided; about 1 cm long and 0·5 cm



Figures 51–54. *Plerandra vitiensis* (Seem) Bailon. **51.** Abaxial view of a leaf with galls. **52.** A single gall enlarged. **53.** Vertical section of a gall. **54.** T.S of gall. (LC, Larval chamber; NZ, nutritive zone; Os, ostiole; VS, vascular strands).

thick; sometimes the midrib is also galled, forming fusiform hypophyllous swellings.

The gall consists of fleshy, ovoid hypophyllous pouch and a minute conical epiphyllous covering growth. The ostiole at the tip of the covering growth leads to a canal like larval cavity, which is irregular in outline in transection. The cells immediately surrounding the larval cavity, especially in the upper part, show centripetal meristematic activity, as a result of which some interior cells are crushed. The gall parenchyma consists of thin-walled highly hypertrophied undifferentiated compact parenchyma polygonal cells outside, and radially elongated in the interior; cells generally diminish in size outwards; cells lining larval cavity collapsed and the cells of the meristematic zone do not contain any special inclusions. The vascular bundles at the periphery dilated due to the hyperplasia and hypertrophy of the parenchyma cells. However, the different components of the vascular bundles are distinct and the tracheary elements remain normal. The gall is 6 times thick as the normal leaf and the bulk of the gall tissue is derived from the spongy mesophyll tissue.

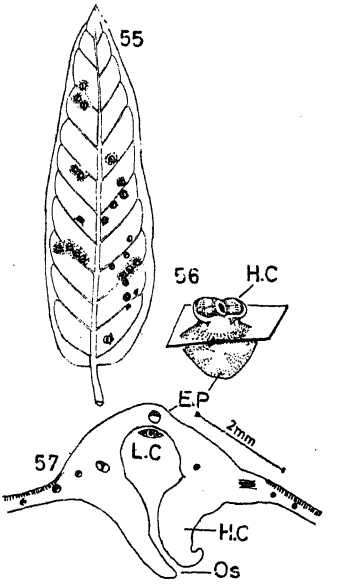
Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

14. Family Myrsinaceae

14.1 *Maesa tabacifolia Mez.* (*figures* 55–57)

Gall No. 833 Psyllidae

Epiphyllous, scattered, surrounded by a chlorotic halo; with large epiphyllous hemispherical pouch and a small hypophyllous conical covering growth; the cover-



Figures 55–57. *M. tabacifolia* Mez. **55.** Abaxial view of the leaf with galls. **56.** An entire gall enlarged. **57.** Vertical section of gall. (EP, Epiphyllous pouch; HC, hypophyllous covering growth; LC, larval chamber with larva; Os, ostiole).

ing growth ends with a recurved bilobed lip, enclosing a slit-like ostiole, leading into a large smooth larval cavity with a single larva inside.

The gall tissues show marked deviation from those of the normal leaf. The parenchyma has undergone hyperplasia and hypertrophy in specific planes in addition to hypoplasia at certain regions. In the mature gall, the following 3 distinct regions may be observed.

(i) Transitional region represents the portion of the gall where the lamina merges into the gall proper, where the palisade and spongy tissues are recognisable. The palisade cells, which are normally 2-layered, have divided periclinally resulting in 4–6 layers of small compact cells. The spongy cells are similar to those of the normal leaf; but air cavities and the surrounding cells are much larger. Towards the larval cavity, the spongy mesophyll cells have divided both periclinally and anticlinally and the derivatives have undergone extensive vertical stretching, forming compact elongated cells, lying parallel to the long axis of the covering growth. However, the palisade cells do not show such a stretching; they remain short and compact. The vascular bundle is dilated due to proliferation of parenchyma cells within the bundle.

(ii) The roof of the gall cavity exhibits suppression of usual tissue differentiation; the number of cell layers in this part is doubled as compared to normal leaf. However, the cells are small, compact and uniform; the thickness remains normal.

(iii) The region of covering growth develops entirely from the spongy mesophyll cells by the downward elongation of cells. The cells in this region are thin, compact, elongated parallel to the long axis of the cover cone. The cells lining the gall cavity are small without any cytological features of special interest.

Locality: Viti Levu: Cuvu, Navouvoa Road; Coll. D S Singh, 12 January 1979.

14.2 *Tapeinosperma sp.* (*figures* 58 *and* 59)

Gall No. 847 Cecidomyiidae midge

Scattered, solitary, lenticular gall, with hypophyllous ostiole dark green circular spot; upto 50 galls per leaf; size 2–3 mm in diameter.

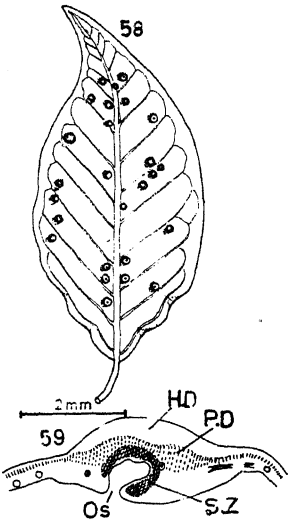
The gall is more than 4 times thicker than the leaf and the gall tissues are formed by proliferation of all ground tissues in varying degrees. The upper epidermal cells divided periclinally at certain regions to form 2–3 layers of cells. The single hypodermal layer of cells is also proliferated to form 4 layers of much hypertrophied cells. The palisade cells are divided in periclinal plane to form 4 layers of cells which are recognisable by the presence of chloroplasts. The spongy parenchyma is divided profusely in all planes and the derivatives are enlarged, circular and more or less compact. The bundle sheath cells are not distinguishable. The larval cavity is wide and is surrounded by 2–3 layers of small empty cells. Following this is the sclerotic zone, which is made up of brachy sclereids.

Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

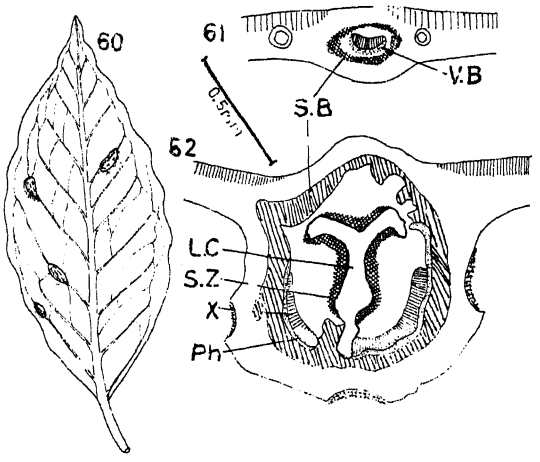
14.3 *Tapeinosperma sp.* (*figures* 60–62)

Gall No. 854 Cecidomyiidae midge

Hypophyllous, small inconspicuous, fusiform or cylindrical, rusty-brown swellings



Figures 58 and 59. *Tapeinosperma* sp. **58.** Abaxial view of the leaf with galls. **59.** Vertical section of a gall (HD, Hypodermal derivatives; Os, ostiole; PD, palisade derivatives; SZ, sclerotic zone).



Figures 60–62. *Tapeinosperma* sp. **60.** Abaxial view of the leaf with vein galls. **61.** T.S. of vein. **62.** T.S. of vein gall. (LC, Larval chamber; Ph, phloem; SB, sclerenchymatous bundle sheath; SZ, sclerotic zone; VB, vascular bundle; X, xylem).

mostly of the lateral veins; covered by membranous scales; fully mature gall measures upto 8 mm long and 1–1·5 mm broad.

In a section of the gall larval cavity is 'Y' shaped, spaced in the centre, which splits the vascular arch into two segments. The parenchyma shows diffuse proliferation and the outermost layers form a suberised periderm of 3–4 layers. The sclerenchyma sheath of the vascular bundle persists around the vascular tissues. A few layers of cells around the cavity are lignified and no nutritive tissue is evident in the mature gall.

Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

15. Family Apocynaceae

Pagiantha thurstonii Seemann (*figures* 63 *and* 64)

Gall No. 864 Cecidomyiidae

Lenticular, pale green gall, scattered on the lamina, larger hypophyllous and thinner epiphyllous swellings, measuring about 2 mm in diameter and 1 mm thick.

There is a single, laterally stretched disc-shaped larval cavity, with one larva. In the region where the normal leaf merges with the gall, the cells show pronounced hypertrophy obliterating the air spaces. In the region of the gall proper, parenchyma undergone extensive periclinal divisions, giving rise to vertical tiers of cells; these cells smaller with dense contents around the larval cavity, and highly vacuolated and vertically stretched away from it, especially so in the abaxial part of the gall. Thus, the gall consists of several layers of compact cells, without histological distinction; lower epidermis ruptured due to stretching in surface plane.

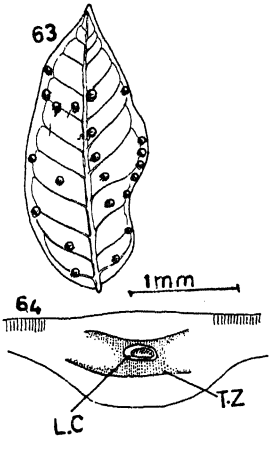
Locality: Taveuni: Qeleni Road; Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.

16. Family Convolvulaceae

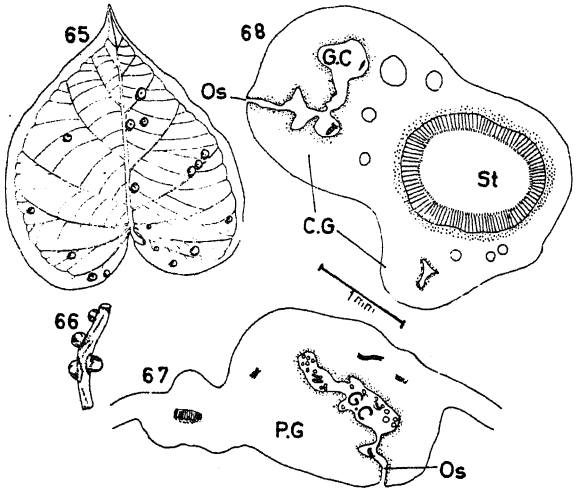
16.1 *Ipomaea gracilis R. Br.* (*figures* 65–68)

Gall No. 872 *Eriophyes* sp.

Epiphyllous or hypophyllous globose pouch gall on leaf and covering-growth gall on the petiole and stem; scattered and solitary, measuring about 1–2 mm high and 1 mm



Figures 63 and 64. *Pagiantha thurstonii* Seemann. **63.** Abaxial view of the leaf with galls. **64.** Vertical section of a gall. (LC, Larval chamber with larva; TZ, tiered zone of cells).



Figures 65–68. *I. gracilis* R. Br. **65.** Abaxial view of a leaf with galls. **66.** Stem galls. **67.** Vertical section of a leaf gall. **68.** T.S. of stem gall. (CG, Covering-growth gall; GC, gall chamber with mites; Os, ostiole; PG, pouch gall; St, stem portion).

in diameter; covering-growth galls are slightly larger than the foliar galls; both yellowish-green glabrous and fleshy.

The foliar gall has a hemispherical pouch portion and an equally projecting covering-growth portion, with the ostiole at its summit; the ostiole leads into a narrow and curved passage, ending in the irregular gall cavity with fleshy emergences. The gall consists of compact layers of homogenous parenchyma cells; one or two layers of cells bordering the gall cavity destroyed and form a dark lining layer all around the cavity.

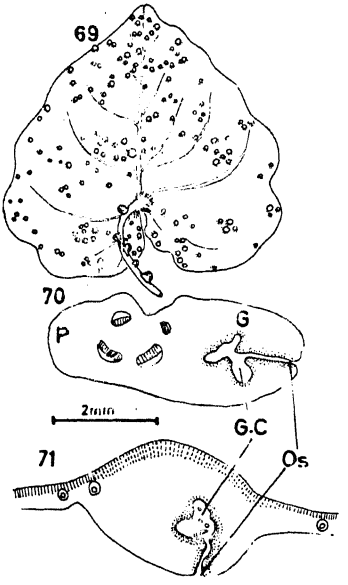
The stem and petiole galls develop by proliferation of the cortical tissue, the vascular tissues not affected; from the cortex develops a small bowl-shaped structure, which later grows into a spherical body with the ostiole at the summit. The gall cavity irregular due to development of the fleshy emergences. The gall tissue consists of large compact irregular and variable parenchyma cells. The cells around the gall cavity are smaller and densely cytoplasmic. The bordering cells are destroyed, forming a dark lining around the cavity as in the leaf gall. The laticiferous ducts of the gall are larger in size and more in number than those of the normal organ.

Locality: Taveuni: Lavena; Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.

16.2 *Ipomaea sp.* (*figures* 69–71)

Gall No. 830 *Eriophyes* sp.

Epiphyllous or hypophyllous, small, hemispherical galls about 2 mm large on leaf, stem and petiole, scattered and numerous, more than hundred per leaf; each gall has a hemispherical pouch on one side and a minute conical covering growth on the



Figures 69–71. *Ipomaea* sp. **69.** Abaxial view of the leaf with foliar and petiole galls. **70.** T.S. of petiole gall. **71.** Vertical section of foliar gall. (G, Gall part; GC, gall chamber; Os, ostiole; P, petiole).

opposite side, with narrow ostiole at the tip, leading to the gall cavity, in which several fleshy emergences project from the surface. On the stem and petiole, the galls are small covering growth with the ostiole at the summit of the outgrowth.

16.2a *Foliar gall*: The tissues of the foliar gall differ strikingly from those of the normal leaf. The outer epidermis of the gall is continuation of the upper epidermis of lamina; cells are slightly laterally expanded, but retain the tannin contents. The palisade cells have undergone periclinal divisions, increasing the number of layers upto seven of irregular cells, not arranged in rows as in the normal portion. These cells form a narrow outer zone of the domed part of the gall. The spongy cells form the major part of the gall and show two distinct zones. About 4 layers of cells around the gall cavity show pronounced anticlinal divisions and their derivatives allign themselves in regular semi-circular rows. Outer to this zone are larger, irregular, less compact cells without regular allignment. These cells are largely hypertrophied. The cells in the covering growth are uniformly elongated parallel to the long axis of the gall. The lower epidermis, which is the seat of attack by the mites, consists of densely cytoplasmic cells with prominent nuclei. In the older galls, this layer is destroyed by the excessive feeding activity of the mites and the subepidermal cells resemble the changed epidermal cells.

16.2b *Petiole and stem galls*: In these cases, only the cortical cells take part in the formation of the galls and the vascular tissues are least involved. The parenchyma cells of the cortex proliferate around the point of infection to produce a globular covering growth. The ostiole at the summit of the covering growth leads to the gall

cavity. The gall tissue consists of compact much dilated parenchyma cells. The lining layer of the gall cavity consists of distinct cells with dense cytoplasm and prominent nuclei, as those of the foliar gall.

Locality: Viti Levu: Deuba Beach; Coll. M S Mani, Sundaresan and M K Kamath, 12 December 1978.

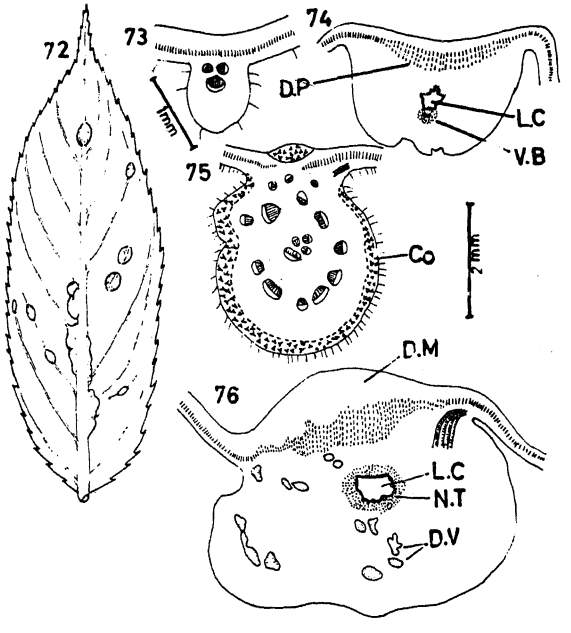
17. Family Gesneriaceae

Cyrtandra sp. (figures 72–76)

Gall No. 839 Cecidomyiidae Midge

Hypophyllous, fusiform or irregular exclusive fleshy, soft, solid, indehiscent and yellowish-green swellings of the midrib, lateral veins and veinlets; sometimes, unilateral bulging of the midrib; the galls do not project above; measurement 6 mm thick and 1–2 cm long.

The midrib gall has a single centrally situated larval cavity. The vascular bundles are scattered among the much dilated, irregular parenchyma cells, containing sphaeroraphides; collenchyma tissue found in the outer region of the normal vein is not differentiated in the gall. In the adaxial portion of the gall, the cells of the multiple epidermis and palisade tissue have undergone periclinal divisions, increasing in the



Figures 72 – 76. *Cyrtandra sp.* **72.** Abaxial view of the leaf with vein galls. **73.** T.S. of a lateral vein. **74.** T.S. of lateral vein gall. **75.** T.S. of a midvein. **76.** T.S. of midvein gall. (Co, Cortex; DM, derivatives of multiple epidermis; DP, derivatives of palisade tissue; DV, distorted vascular strands; LC, larval chamber; NT, nutritive tissue; VB, vascular bundle).

number of layers. The gall on the lateral vein also has a single layer larval cavity. Vascular bundles collapsed and consist of xylem elements in the middle of disintegrated phloem tissue. The multiple epidermis and the palisade tissue divided in periclinal plane and the ground parenchyma has also divided, giving rise to irregular compact cells. In the lateral vein gall the place of the vascular bundle is occupied by the larval cavity. The hypodermal as well as the palisade cells have increased in radial plane and the parenchyma cells of the abaxial part have multiplied forming large irregular cells.

Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.

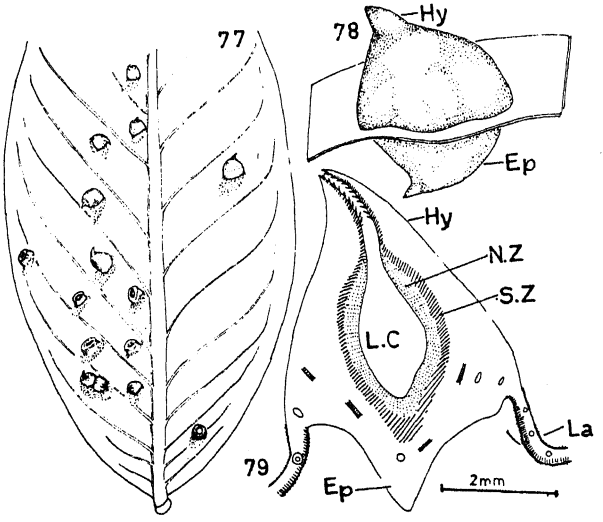
18. Family Myristicaceae

18.1 *Myristica macrantha A C Smith (figures 77–79)*

Gall No. 843 Psyllidae

A large hypophyllous dome-shaped, beaked covering-growth about 4 mm and a smaller epiphyllous conical, curved pouch about 3 mm long; hard, brittle, smooth, bright reddish-brown; mature gall dehisces by a circular line along the subbasal part and falls off as a lid, leaving behind the epiphyllous portion as a cup; median part of the gall 5 mm wide.

Each gall consists of two distinct parts: a larger hypophyllous dome-shaped part with a small beaked tip; the ostiole occurs at the summit of the beak. The epiphyllous



Figures 77 – 79. *Myristica macrantha* A. C. Smith. **77.** Abaxial view of the leaf with some intact and some dehisced galls. **78.** One entire gall enlarged. **79.** Vertical section of a gall. (Ep, Epiphyllous portion of the gall; Hy, hypophyllous covering growth portion; La, lamina; LC, larval chamber; NZ, nutritive zone; SZ, sclerotic zone).

part is hemispherical with a short sharply curved beak. The ostiole leads to the elliptical axial smooth wide larval cavity, surrounded by several layers of small rectangular nutritive cells filled with dark contents; followed by a broad zone of sclereids, which encircles the entire gall cavity and extends upto the path of the ostiole. External to the sclerotic zone, the gall tissue consists of homogenous layers of irregular thin-walled cells, stretched tangentially to the plane of the blade and represent apparently derivatives of the exaggerated palisade tissue; vascular strands do not extend into the hypophyllous part of the gall, but occur as small islands in the epibasal portion only. All gall cells, including the sclereids, contain spherical globules of tannin. The passage of the ostiole is clothed with minute trichomes.

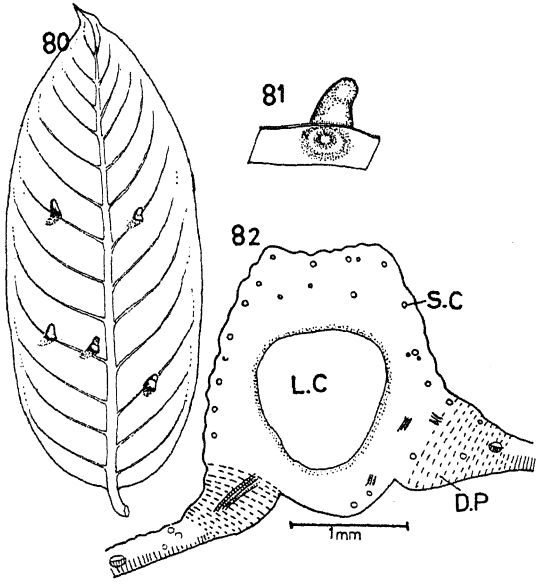
Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

18.2 *Myristica sp.* (*figures* 80–82)

Gall No. 861 Psyllidae

Hypophyllous, cylindrical and pyramidal, scattered, sparse, dark brown, closed, indehiscent pouch gall; marked by a circular epiphyllous depression and a central bluntly conical spot; each gall measures about 3 mm long and 1 mm broad.

In vertical section the gall appears as a pyramidal structure, with flat apex and wavy surface, with large, smooth, obovoid axial larval cavity; gall tissue consists of homogenous layers of compact, thin-walled highly tanniniferous cells of varying size and shape. A few layers of cells around the larval chamber possess small spherical



Figures 80–82. *Myristica* sp. **80.** Abaxial view of the leaf with galls. **81.** One gall enlarged. **82.** Vertical section of the gall (DP, Derivatives of palisade tissue; LC, larval chamber; SC, secretory canal).

starch grains. The secretory cavities similar to those of the normal leaf are found in the peripheral region of the gall. The vascular traces do not extend beyond the middle of the gall. The outer boundary of the gall is formed by a distinct, cuticularised epidermis.

Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

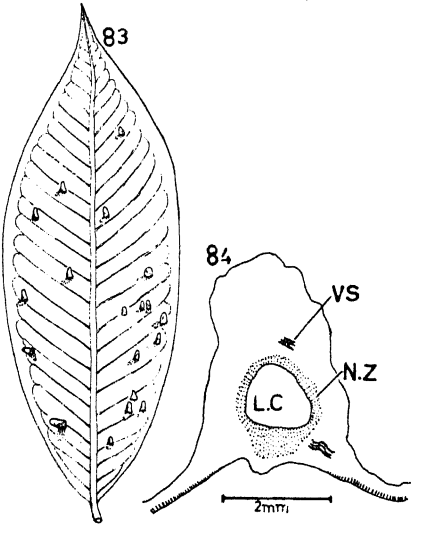
19. Family Lauraceae

Cryptocarya sp. (figures 83–84)

Gall No. 842 Psyllidae

Hypophyllous, conical or subtruncate, pyramidal, sessile, indehiscent, smooth, reddish-brown, solitary and scattered; 3·5 mm long and 2 mm thick; ovoid gall cavity is basal, the upper part being solid; a minute truncated projection occurs on the opposite side of the gall. The gall tissue consists of homogenous, compact, thin-walled, polygonal, tannin-filled, parenchyma cells. A few layers of cells around the gall cavity are smaller and darker. Some of the cells are much elongated and canal-like, with granular contents. The lining layer of the gall cavity consists of small rectangular cells. The outer epidermis differs from that of leaf in being less papillate. The vascular tissues of the leaf extend into the gall upto the upper solid part.

Locality: Taveuni: Qeleni Road; Coll. M S Mani, Sundaresan and M K Kamath, 20 December 1978.



Figures 83–84. *Cryptocarya* sp. **83.** Abaxial view of the leaf with galls. **84.** Vertical section of a gall. (L C, Larval Chamber; N Z, nutritive zone; V S, vascular strand).

20. Family Euphorbiaceae

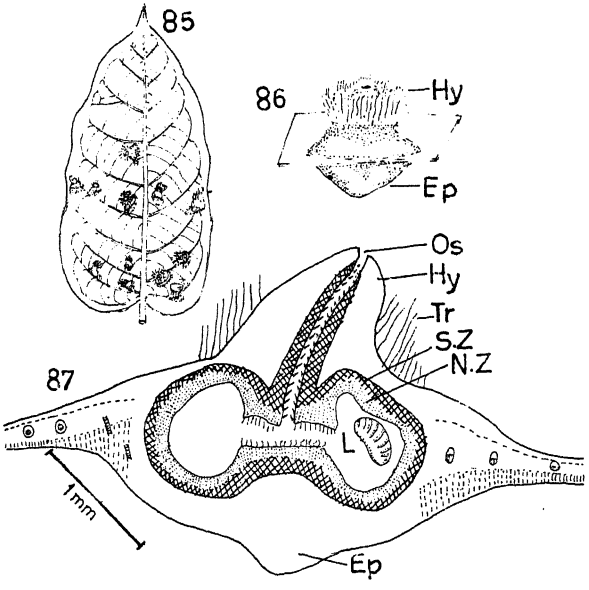
20.1 *Glochidion ramiflorum J R and G Forst.* (*figures* 85–87)

Gall No. 836 Cecidomyiidae

Hypophyllous, solitary, scattered, hairy, indehiscent, two-chambered, conical covering growth gall, 3 mm high and 3 mm wide, with the ostiole on the summit of the minute hypophyllous covering growth; the gall is marked above by a smooth, circular ridge.

Erect or curved, conical covering growth, with an apical ostiole; surface studded with long, uniseriate, unbranched, multicellular hairs, which almost mask the gall. The epiphyllous part is a glabrous hemispherical pouch. Ostiolar canal with straight, stiff outwardly directed hairs; spaceous gall cavity is divided into 2 incomplete, laterally disposed chambers by short, thick, pillar-like outgrowths from the floor and by pendulous tissue from the roof of the gall cavity, meeting each other, but do not fuse, so that the incomplete central partition divides the gall cavity into lateral, spherical empty atrium and a larval chamber proper.

The gall cavity is surrounded by 7 or 8 layers of thin-walled and polygonal nutritive cells; followed by 4 or 5 layers of brachysclereids, extending upto the ostiole through the ostiolar canal. External to the sclerotic zone are several layers of parenchyma cells. In the region of the adaxial pouch, these parenchyma cells are compact and polygonal. In the basal region of the conical portion, they are less



Figures 85–87. *G. ramiflorum* J.R. and G. Forst. **85.** Abaxial view of the leaf with galls. **86.** One gall enlarged. **87.** Vertical section of gall (Ep, Epiphyllous part of the gall; Hy, hypophyllous conical part of the gall; L, larva; Os, ostiole; NZ, nutritive zone; SZ, sclerotic zone; Tr, trichome).

compact, vertically stretched and gradually merge into the different tissues of the leaf. The surface cells of the central pillar and of the pendent mass elongate into straight, stiff, unbranched unicellular hairs. The annular sclereids of the mesophyll are considerably hypertrophied and much elongated along with other cells of the gall.

Locality: Viti Levu: Cuvu, Novouvou Road; Coll. M S Mani, M K Kamath and Sundaresan, 12 January 1979.

20.2 *Macaranga magna Turrill* (*figures* 88 *and* 89)

Gall No. 831 *Eriophyes* sp.

Epiphyllous, shallow, wide open, blister-like pouches, about 7 mm large; often very much crowded; young galls circular and pale green; mature ones irregular and brown, with soft and velvety dense tomentum.

The gall consists of considerably hypertrophied epidermal cells. The upper epidermal cells divided in anticlinal plane and stretched laterally, thus accommodating the increased surface area of the gall. The palisade cells widened several times, accompanied by reduction in the height of the cells and they remain single layered. The spongy mesophyll cells have also undergone considerable hypertrophy thus obliterating the intercellular space. The hypertrophy of different tissues leads to the arching over the lamina to form the shallow pouch. Dense unicellular, unbranched epidermal hairs occur on both outer and inner surfaces of the gall curled, with granular inclusions.

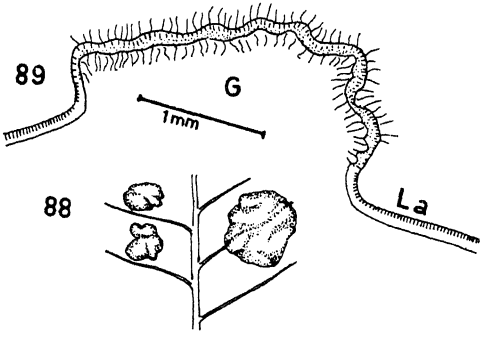
Locality: Viti Levu: Deuba beach side; Coll. M S Mani, Sundaresan and M K Kamath, 12 December 1978.

21. Family Ulmaceae

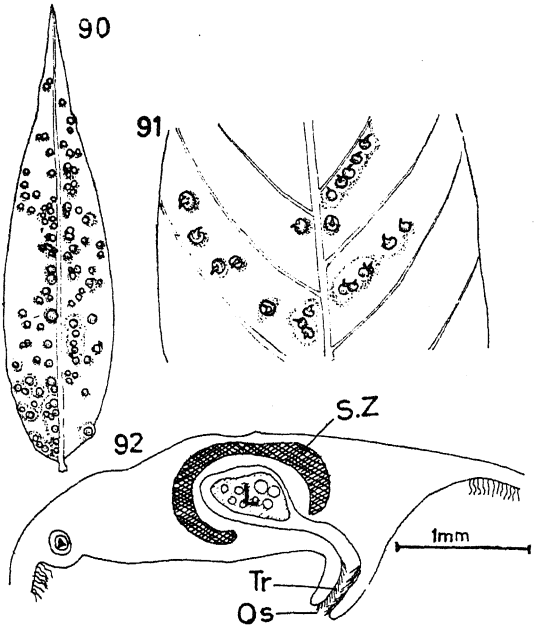
21.1 *Gironniera celtidifolia Gaud.* (*figures* 90–92)

Gall No. 857 Cecidomyiidae midge

Hypophyllous, solitary, scattered or agglomerate, indehiscent, glabrous, reddish-brown pouch gall, with a circular raised spot above and is a minute curved beak-like



Figures 88 and 89. *Macaranga magna* Turrill. **88.** A portion of the leaf with galls. **89.** T.S. of leaf through gall (G, Galled portion; La, lamina).



Figures 90–92. *G. celtidifolia* Gaud. **90.** Adaxial view of the leaf with galls. **91.** Abaxial view of the galls. **92.** Vertical section of the gall (L, Larva; Os, ostiole; SZ, sclerotic zone; Tr, trichome).

hypophyllous covering growth, about 4 mm in diameter and 2 mm high in the median part.

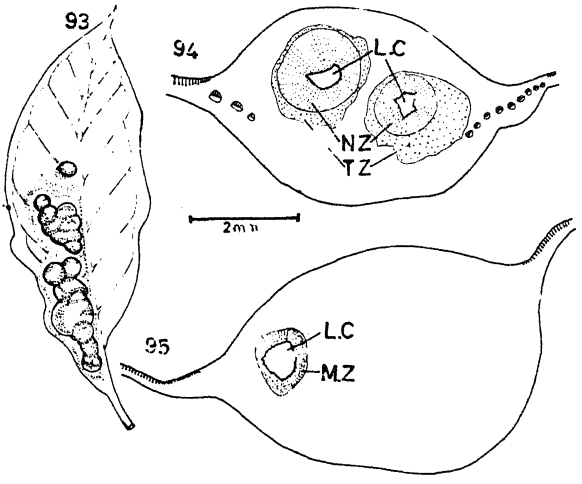
The gall is more or less fusiform in cross section; larval cavity horizontally elongated in the middle with a single larva; ostiolar canal horizontal for a short distance then bent down to ostiole, situated at the tip of the small pointed, excentric covering growth. The passage in the region of the covering growth is filled by dense, brownish, straight hairs, directed downwards, and thicker and sturdier than those on the leaf. The mass of gall is of compact thin-walled homogenous polygonal cells; in the roof of the larval cavity, the upper epidermis is immediately followed by 5–7 layers of brachysclereids, which form a horseshoe-shaped zone around the larval chamber; inner to the sclerotic zone are several layers of laterally stretched parenchyma cells. The gall tissue contains large and dense starch grains and most cells are tanniniferous.

Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.

21.2 *Gnetum gnemon Linn.* (*figures* 93–95)

Gall No. 848 Cecidomyiidae

Foliar gall; blister-like, yellowish-green, smooth indehiscent, solitary or agglomerate, closed gall, more hypophyllous than epiphyllous; lamina depressed around the gall,



Figures 93–95. *G. gnemon* Linn. **93.** Abaxial view of the leaf with gall type 851. **94.** Vertical section of gall No. 851. **95.** Vertical section of gall No. 848 (L C, Larval chamber; M Z, meristematic zone; N Z, nutritive zone; T Z, thick walled cell zone).

so that the gall appears as if it arises out of a pit. The gall tissue consists of highly proliferated and hypertrophied cells. One or more large, irregular larval cavities occur either at the centre or peripherally in the gall; surrounded by a thick layer of collapsed cells; followed by a meristematic zone; asterosclereids scattered in the gall tissue; they are more numerous and larger than in normal leaf; gall stomata epiphyllous; hypophyllous galls display certain modifications as compared to the normal leaf stomata; subsidiary cells of the gall stomata proliferated, losing their identity and the epidermal cells have also highly hypertrophied.

Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

Gall No. 851 (figures 93 and 94) Cecidomyiidae midge

Foliar gall; irregular, blister-like, solitary or agglomerate, closed, indehiscent, smooth, yellowish-green, pocken-gall, projecting more or less equally on both surfaces of the blade; 7 mm wide and 4 mm thick; more or less elliptical or circular in transection with 1 or 2 larval cavities in the middle; cavities small spherical, surrounded by wide concentric zone of thin walled, compact, polygonal nutritive tissue, with prominent nuclei and numerous starch grains; outer of this zone is the parenchyma zone with similar, but slightly thick walled, empty cells; delimited by a distinct epidermis, cells of which have undergone anticlinal divisions and lateral expansion; asterosclereids are scattered in the periphery of the gall and they resemble those of the normal leaf except for a slight increase in size.

Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

22. Family Filicales

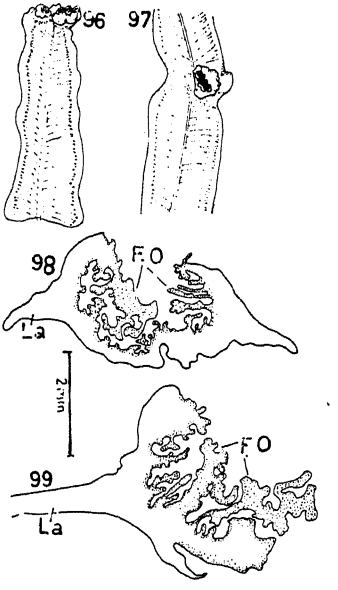
Nephrolepis biserrata (Sewartz) Schott. (figures 96–99)

Gall No. 863 *Eriophyes* sp.

Hypophyllous, cup-shaped, sessile or subsessile, covering-growth of irregular size and shape occurring distally on the margins, or on the midrib of the pinnule; bowl-shaped gall has a wide opening, the rim of the cup being fringed and incurved. The outer surface of the gall is smooth and the interior is brownish. Measurement 2 mm thick and 4 mm broad.

The gall develops as a covering growth from the lower epidermis and a few layers of the subepidermal ground tissue to assume a fleshy hollow cup-like structure. The cells of inner lining layer of the cup grow into many irregularly branched filaments or thin lobed plates, one cell thick; plate-like emergences several cells thick at base and ultimately become uniseriate at the tip; plates and filaments fill up the entire interior of the gall and mites crowded in the narrow spaces of these outgrowths; cells of the emergences filled with tannin, the characteristic cytological features of the lining layer of cells, usually observed in most of the mite galls, are not evident here. The cells constituting the gall are much hypertrophied, irregular in shape, loosely arranged and possess thin wavy walls.

Locality: Taveuni: Qeleni; Coll. M S Mani, M K Kamath and D S Singh, 20 December 1978.



Figures 96–99. *Nephrolepis biserrata* (Sewartz) Schott. **96.** Pinnule with terminal galls. **97.** Pinnule with cup shaped gall on the midrib. **98.** Vertical section of the gall in the middle portion of lamina. **99.** Vertical section of gall at the apex of lamina. (La, Lamina; PO, plate-like outgrowths).

23. Species Incertae sedis

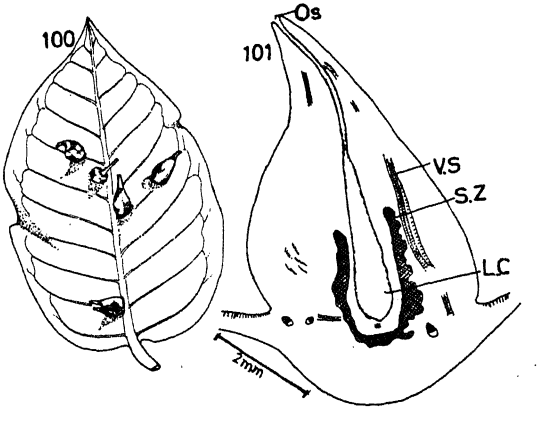
Galls on unidentified plants (figures 100 and 101)

Gall No. 845 Cecidomyiidae midge

Hypophyllous, flask-shaped, fleshy, solitary and sessile; erect or curved; with a dilated basal part measuring about 3 mm in diameter and an apical part produced into narrow cylindrical slender, beaked straight or somewhat curved horn-like process, 1 mm diameter; gall measures 7 mm high from base to the tip; on the upper surface of leaf the basal part of gall forms a hemispherical raised spot, ostiole occurs on the summit of the beaked horn-like process; exit hole at the dilated basal part of the gall.

The gall develops as a small conical hypophyllous covering growth, the gall cavity in the initial stage is filled with club-shaped, glandular outgrowths from the surface of the cavity; a few hairs are seen at the ostiolar region, in the mature gall glands and trichomes absent in ostiole; gall cavity axial and it runs upto the tip of the gall, is wide at base and narrow and canal-like inside the horn; the basal wider part of the larval cavity is surrounded by a dark layer of collapsed cells, inside which follow a zone of wound healing tissue and a broad zone of sclereids; gall fully vascularised, vascular strands extend upto the extreme tip of the gall; path of the exit hole is also surrounded by the wound healing tissue; cells bordering the narrow passage of the larval cavity, leading to the ostiole are intact, thin-walled and elongated parallel to the canal. Outer to the sclerotic zone occurs the vascular strand followed by several layers of homogenous compact polygonal cells, which become less compact and irregular towards the periphery; epiphyllous hemispherical projection also consists of compact homogenous mass of cells.

Locality: Viti Levu: Novouvou Road; Coll. D S Singh, 12 January 1979.

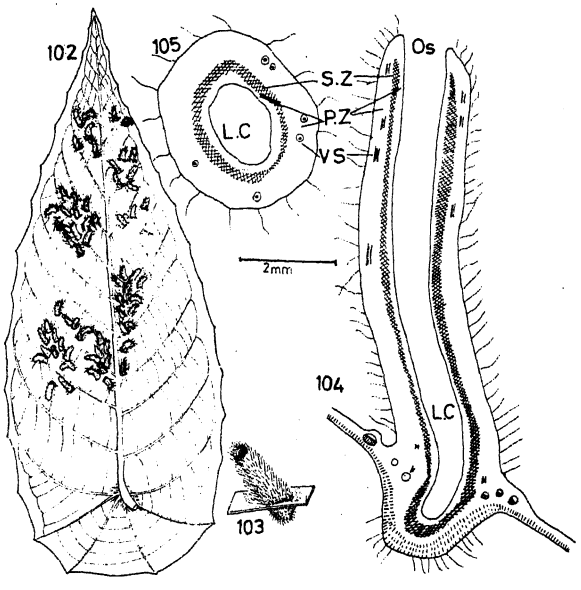


Figures 100 and 101. Gall No. 845. An unidentified plant. **100.** Leaf with galls. **101.** Vertical section of gall. (LC, Larval chamber; Os, ostiole; SZ, sclerotic zone; VS, vascular strand).

Gall No. 846 (figures 102–105) Cecidomyiidae midge

Hypophyllous, cylindrical, straight or lopsided, scattered covering-growth gall, measuring about 1 cm long and 2 mm in diameter; with a hemispherical epiphyllous pouch; the entire outer surface of the gall covered by straight, rough, bristle-like, unicellular, unbranched trichomes; a circular ostiole occurs at the tip of the cylinder.

The gall develops as a small conical hypophyllous covering growth with a minute epiphyllous projection. The covering growth is derived entirely from the spongy tissue and consists of small compact transversely dividing cells, arranged in regular rows parallel to the vertical axis of the young gall; ostiole very narrow, outer surface covered by trichomes; a few layers of cells in the outer zone of the young gall do not show much divisions and the cells are somewhat irregular; epiphyllous projection is formed by periclinal divisions of the adaxial epidermis and the palisade cells. In the mature gall, the cells have extensively elongated in a vertical plane at right angles to the leaf surface, forming the cylindrical body of the gall. As the elongation stops, a few layers of cells inner to third or fourth layers from the larval cavity differentiate into sclerotic zone, which lines the entire larval cavity from the base upto the tip of the gall. Outer to the sclerotic zone occurs the vascular strands which extend to the extreme end of the cylinder and these strands are extensions of the vascular system of the lamina. A few layers of cells, inner to the outer epidermis, which are derived from the cells of less meristematic activity at the initial stage of the gall, are filled with tannin. The epidermal hair has a bulbous base, which is deeply embedded below the epidermal layer. In the mature gall, the ostiole becomes wider and circular through which the insect escapes.



Figures 102–105. Gall No. 846. An unidentified plant. **102.** Abaxial view of leaf with galls. **103.** A single gall enlarged. **104.** Vertical section of gall. **105.** T.S. of gall. (LC, Larval chamber, Os, ostiole; PZ, parenchyma zones; SZ, sclerotic zone; V S, vascular strands).

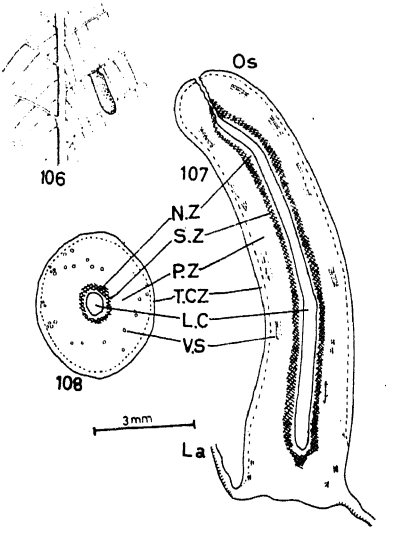
Locality: Viti Levu: Colo-i-Suva; Coll. M S Mani, M K Kamath and Sundaresan, 25 January 1979.

Gall No. 871 (figures 106–108) Cecidomyiidae midge

Hypophyllous covering growth gall, occurring mostly near the veins, thick, quite conspicuous, cylindrical, sessile, greyish-green, softly hairy, indehiscent, straight or slightly curved; ostiole minute and terminal, 1·5 cm long and 0·5 cm thick; the gall develops as a small covering growth on the abaxial side by proliferation of mesophyll; cells of the palisade and spongy mesophyll tissues divide repeatedly by periclinal walls and give rise to a hemispherical mass of small cells filled with dense cytoplasmic contents. These cells stretch in vertical plane at right angles to the surface of the leaf and develop into a long cylindrical gall with the ostiole at the tip. The ostiole leads to the narrow straight canal-like axial larval cavity which extends down to the base of the cylinder.

The mature gall has 4 distinct zones of tissues. The gall cavity is surrounded by a zone of radial files of regular rectangular cells, the cells bordering the cavity are collapsed. Outside of this lies a sclerotic zone of either angular or circular cells with secondary wall thickness gradually diminishing outwards; outside of this is a wide zone of large thin-walled parenchyma, containing large concentric starch grains; vascular tissues occur in this zone as narrow as anastomosing strands. The outermost zone is made up of somewhat thick-walled, smaller cells, the lateral walls of which possess circular primary pits. A distinct epidermis delimits the fourth zone. Simple and stellate hairs occur on the surface of the gall.

Locality: Taveuni: Soqulu Estate; Coll. M S Mani, M K Kamath and D S Singh, 19 December 1978.



Figures 106–108. Gall No. 871. An unidentified plant. **106.** A portion of lamina with a gall. **107.** Vertical section of a gall. **108.** T.S. of gall. (La, Lamina; LC, larval chamber; NZ, nutritive zone; PZ, parenchyma zone; SZ, sclerotic zone; TCZ, thick walled cell zone; V S, vascular strands).

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Biochemical correlates in *Rhipiphorothrips cruentatus—Terminalia catappa* interactions with special reference to leaf infestation patterns

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Abstract. Leaf age correlated infestation patterns of *Rhipiphorothrips cruentatus* on *Terminalia catappa* revealed specificity in stage and site selection. An indepth analysis of the quantitative as well as qualitative profiles of proteins, amino acids and fatty acids in age specific leaves exemplified a correlatable influence on the infestation patterns.

Keywords. *Rhipiphorothrips cruentatus*; *Terminalia catappa*; biochemical correlates; site selections; host selections.

1. Introduction

Phytophagous thrips species tend to selectively exploit certain areas of leaves of different ages (Fennah 1963) and also show vertical stratification restricted to different nodes (Ananthakrishnan 1984; Ananthakrishnan *et al* 1982), in order to locate nutrient rich solutes at specific sites. Infestation is known to decline with the age of the leaves following a reduction in the availability of soluble nitrogen, an aspect exemplified by *Selenothrips rubrocinctus* on *Anacardium occidentale* (Fennah 1963). The amino acid concentration occurring within the leaves of varying age also offers a clue to the ability of the insect to establish themselves on the host. In this connection, the role of free amino acids in host preference has been discussed in relation to *Retithrips syriacus* infesting *Ricinus communis* (Ananthakrishnan and Muraleedharan 1972), as also the allied panchaetothripine species *Rhipiphorothrips cruentatus*, a highly polyphagous species (Ananthakrishnan and Muraleedharan 1974). A reduction in the quantity/quality of food of adult females tends to result very often in the delay and decline in the rate of egg production (Scriber and Slansky 1981). Besides, feeding is strongly influenced by olfactory and gustatory responses resulting from both nutritional and non-nutritional components in the food so that such behavioural responses as change in feeding rates could result from changes in food quality. Though a broad-based idea of the intake of major nutrients like total nitrogen, carbohydrates, proteins and lipids is generally assessed, an indepth analysis of the nature of lipids and amino acids in feeding, growth and development appear essential for a better understanding of the utilization of these substances. An attempt has therefore been made in this paper to analyse the above aspects with reference to *Rhipiphorothrips cruentatus* infesting the myrtaceous tree host *Terminalia catappa*, which shows marked preferences to leaf age.

2. Materials and methods

Based on their position on the branch in relation to the shoot apex, the following different leaf stages (I–V) of *T. catappa* were collected: the axillary/terminal buds

with the I leaves unopened; the II leaves from the terminal bud, partially opened; completely opened and green leaves III from the terminal bud; dark green completely opened IV leaves; and mature V leaves respectively.

Ethanolic extracts of the leaf material were prepared (Harborne 1973), an aliquot of which was acidified with 2 M hydrochloric acid and incubated for 0·5 h, the resultant solution cooled and filtered. The phenols taken into ether from the extract was washed, evaporated to dryness, and taken into ethanol for the estimation of total phenols (Bray and Thorpe 1954) and *o*-dihydric phenols (Johnson and Schall 1952). The ethanol extract devoid of phenols was used for the quantitative estimation of amino acids (Moore and Stein 1948) and carbohydrates (Dubois *et al* 1956). Total chlorophyll was estimated by spectrophotometry of the acetone (80% v/v) extract of the fresh leaves (Yoshida *et al* 1976). Buffer soluble proteins were extracted from the fresh leaves in phosphate buffer saline (pH 7·2, 0·1 M with 0·85% sodium chloride) and estimated (Lowry *et al* 1951). Total lipids were extracted from fresh leaves in $CHCl_3 : CH_3OH$ (2:1) by the method of Folch *et al* (1957) and estimated by gravimetry.

Five g of fresh leaves were ground in ice cold acetone using a prechilled mortar and pestle, and later using an ultrasonic disintegrator. The homogenate was centrifuged at 12500 g for 30 min, at 5°C using a Hitachi refrigerated centrifuge. The residue free of pigments was used for enzyme analysis. 200 mg of the acetone powder was suspended in 4 ml of buffer [phosphate buffer, pH 7·0, 0·3 M for peroxidases; borate buffer, pH 8·8, 0·1 M for phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)] and extracted for an hour at 4°C. The extract was centrifuged at 11500 g for 30 min and the clear supernatant was used for enzyme assay. Peroxidase was assayed (Loebenstein and Linsay 1961) using 2·5 ml of pyrogallol (0·1%), 0·1 ml of 0·1% hydrogen peroxide and 0·1 ml of the enzyme extract and the absorbance at 420 nm was taken at an interval of 30 s for 10 min, using a Hitachi UV-Vis Spectrophotometer. Increase in absorbance by 0·01/min/ml of enzyme used/mg protein was expressed as a specific unit. For the estimation of PAL and TAL (Higuchi 1966), 2 ml of the substrate (0·1% L. phenylalanine and L. tyrosine respectively) was incubated with 1 ml of the enzyme in borate buffer (pH 8·8, 0·1 M) and 1 ml of distilled water for 1 h. 0·4 ml of 3 N HCl was added to stop the reaction and the reaction mixture was twice extracted with ether and dried. The residue was redissolved in 2·5 ml of 0·05 N NaOH and read at 268 nm for PAL and 333 nm for TAL respectively. Specific units were expressed as μg trans.cinnamic acid released/ml enzyme used/mg protein for PAL activity and as increase in absorbance by 0·1/ml enzyme used/mg protein for TAL activity. For the analysis of the qualitative profiles of amino acids in different leaf stages, Hewlett Packard High Performance Liquid chromatography was used. *o*-Phthalaldehyde derivatives of the amino acids in the alcoholic extract was chromatographed (Lindroth and Mopper 1979) using Hypersil ODS 5 μm column. 0·1 M phosphate buffer pH 7·7 and methanol were used as mobile phase and fluorescent derivatives were detected at 340 nm. The amino acids were identified based on standard chromatograms of individual amino acids and the area per cent of individual amino acid peaks were calculated.

To the total lipids extracted from the leaf tissue, 10 ml of ethanol, 3 ml of 28% ammonium hydroxide, 25 ml of petroleum ether and 25 ml of diethyl ether were added to a separating funnel, shaken for 5 min and allowed to stand for 20 min. The bottom phase was drained off, the ether phase was dried to which 3 ml of 0·5 N

NaOH in methanol was added and heated in a steam bath for 15 min. To this, 5 ml of water was added and 2 N HCl were added slowly until the pH was approximately 2. The fatty acid methyl esters were then extracted into 5 ml of petroleum ether and 5 ml of diethyl ether from the acidified methylated lipid extract. Fatty acids were analysed by a Hewlett Packard high performance liquid chromatograph (HPLC) system at two detection wavelengths, i.e. 210 nm and 230 nm using an Hypersil ODS 5 μm column with water and acetonitrile as mobile phase at a flow rate of 0·45 ml according to the gradient programme as per Schuster (1985). Retention times and area per cent of fatty acid methyl esters were recorded.

Polyacrylamide gel electrophoresis (PAGE) was carried out (Davis 1964) using 7·5% gel on a slab gel electrophoretic apparatus in an alkaline buffer system (Tris-glycine buffer, pH 8·6). Acetone powder was suspended in borate buffer and the resultant supernatant was used for qualitative profiles of proteins. 0·02% Coomassie brilliant blue G250 in methanol, acetic acid and water (25:7:68) was used for staining the gels. Zymograms were drawn from the protein stained gels.

3. Results

R. cruentatus, a panchaetothripine species, infests the abaxial side of the leaves of *T. catappa* during the months of March–September. Infestation in terms of number of leaves attacked in several tree branches examined during the above period showed the maximum incidence to be around 56% and the feeding index (which is the area damaged in cm^2/total laminar area in $cm^2 \times$ number of individual thrips) to be $34{\cdot}4 \pm 8{\cdot}24$. The infestation ratio, which is the area of damage of laminar surface/total laminar area in cm^2 was found to be $0{\cdot}24 \pm 0{\cdot}21$ for March–April, $0{\cdot}23 \pm 0{\cdot}14$ for May, $0{\cdot}23 \pm 0{\cdot}13$ for July and $0{\cdot}25 \pm 0{\cdot}13$ for August–September during the period of observation. Analysis of the infestation pattern in different leaf stages indicated the infestation ratio and the percentage ratio to be $0{\cdot}21 \pm 0{\cdot}31$ and 53% for the leaf stage III and $0{\cdot}20 \pm 0{\cdot}31$ and 58% for the leaf stages IV and V, while the leaf stages I and II as well as the senescing yellow leaves were not infested by this thrips species.

Observations on the life cycle of the thrips species on *T. catappa* leaves revealed the number of eggs laid by a single female to be varying from 15–50 and the oviposition rate to be 2–6 per day, the total duration of life cycle ranging from 14–28 days inclusive of the preoviposition period, with the individual stages having the following duration in days: preoviposition period $5{-}7 \pm 0{\cdot}4$; I instar $2{\cdot}36 \pm 0{\cdot}39$; II instar $3{\cdot}38 \pm 0{\cdot}41$; prepupa 1 day; and pupa $2{\cdot}20 \pm 0{\cdot}45$ making the total duration to be around 19 days. However, the total duration and fecundity were 21·06 and $25 \pm 2{\cdot}94$, $14{\cdot}11 \pm 0{\cdot}08$ and $36{\cdot}41 \pm 2{\cdot}94$, and $11{\cdot}86 \pm 0{\cdot}09$ and $45{\cdot}53 \pm 0{\cdot}09$ at 25°C and 84% RH, 30°C and 81% RH and 35°C and 74% RH respectively.

Since infestation by *R. cruentatus* was on the flush leaves (stage III) and on subsequent leaf stages, an analysis of the basic biochemical components in relation to the distribution pattern of this species, appeared relevant. Quantitative analysis of chlorophyll of different leaf stages revealed an increasing trend in terms of concentration, the maximum being 2·188 mg/g fresh weight in completely mature leaves. The quantity of carbohydrates which indirectly indicates the functional efficiency of the quantum of chlorophyll present did not reveal much of variation among different leaf stages, though considerably high quantity (6·5 mg/g fresh weight) was noticed in leaves of stages I and III (figure 1a).

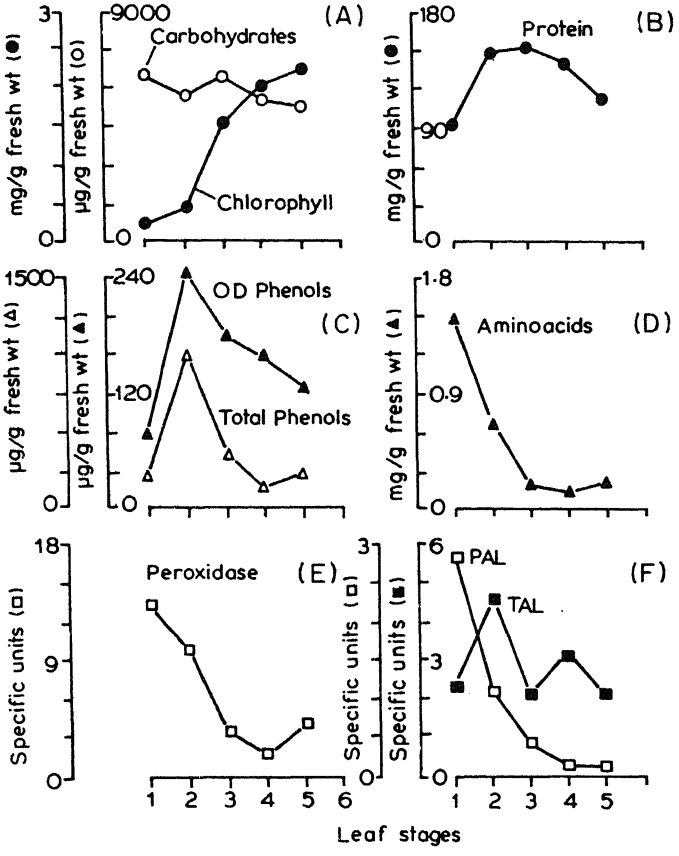

Figure 1. Quantitative biochemical profile in relation to leaf stages of differing age.

Since life-history patterns of herbivores and the associated plants tend to be osely correlated in relation to the availability of nitrogen, investigations on the roteins as well as the free amino acids in the host plants had to be attempted. nterestingly enough, quantitative analysis of proteins revealed again a pattern simi- r to that of carbohydrates in that the maximum quantity was present in leaves of age III (153 mg/g fresh weight) (figure 1b), while the qualitative profile based on AGE revealed maximum number of bands in the extracts of the leaf stage I (figure . Minimum number of bands were found in the III stage leaves indicating the resence of higher concentrations of a few proteins present in the completely open reen leaves. Examination of the free amino acid distribution in the cell sap of the aves of *T. catappa* which might form one of the major components of food for this sect species, revealed a trend of decreasing concentrations in relation to increasing af age (figure 1d). Value as high as 1·525 mg/g fresh weight was recorded for I aves, while the leaf stages III, IV and V did not show much of variation in their uantitative profile, the values being 0·200, 0·125 and 0·225 mg/g fresh weight of ssue analysed.

The qualitative profile of free amino acids and their individual concentrations

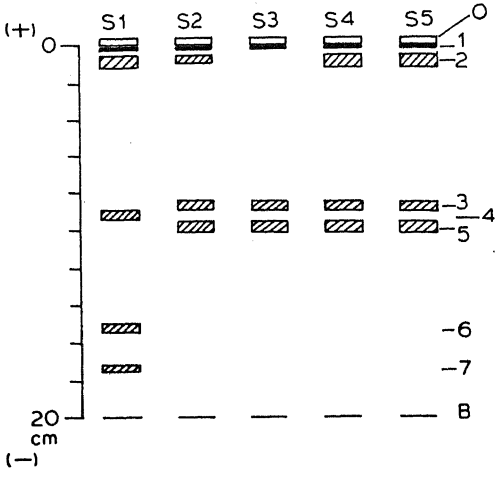

Figure 2. Zymogram of the slab gel electrophoresis of proteins from different leaf stages of *T. catappa*. S1–S5, Indicates leaf stages; B, bromophenol blue; O, origin.

based on the area per cent of the peaks) were examined using a HPLC (figure 3 and able 1). Based on the retention time of standard amino acids, individual amino acids vere detected. In the leaf stage I, the notable amino acids that could not be detected vere threonine, arginine, alanine and valine, and the highest concentrations of total mino acids in this appear to be due to the 10 free amino acids as indicated in table . From stage II onwards, the availability of free amino acids increased in terms of uality, with a minimum of around 14 amino acids inclusive of the known essential mino acids for insect feeding like threonine, arginine, phenylalanine, methionine etc.

While increasing total lipid content was typical with increasing leaf age (figure 4b), HPLC analysis of the fatty acid methyl esters both at 210 nm as well as 230 nm ndicated that decreasing number of fatty acids to be present during increasing leaf ge (figure 4a, table 2). Among the C18 fatty acids, stages I, III and V had linolenic, leic and stearic acids, while stage II had oleic and stearic acids, and stage IV had nly linolenic acid. The fatty acids of differing retention time in the 5 leaf stages are rovided in table 2.

Phenolics which form the product of secondary metabolism of plants, is of interest n insect-plant interactions since they could either be feeding attractants forming part f the food for insects along with other components like proteins, amino acids, arbohydrates etc or as feeding deterrants. Hence, a quantitative study of the total henols, *o*-dihydric phenols and the important enzymes involved in secondary netabolism such as peroxidase, phenylalanine ammonia lyase and tyrosine ammonia yase were also attempted. Decreasing trends in the quantitative profiles of the total henols and the *o*-dihydric phenols (figure 1c) as well as of activities of peroxidase figure 1e), phenylalanine ammonia lyase and tyrosine ammonia lyase (figure 1f) were vident from the leaf stages II–V correlating well with increasing leaf age. However, he terminal buds with I leaves recorded low level of phenolics and comparatively igher levels of activities of all the 3 enzymes studied.

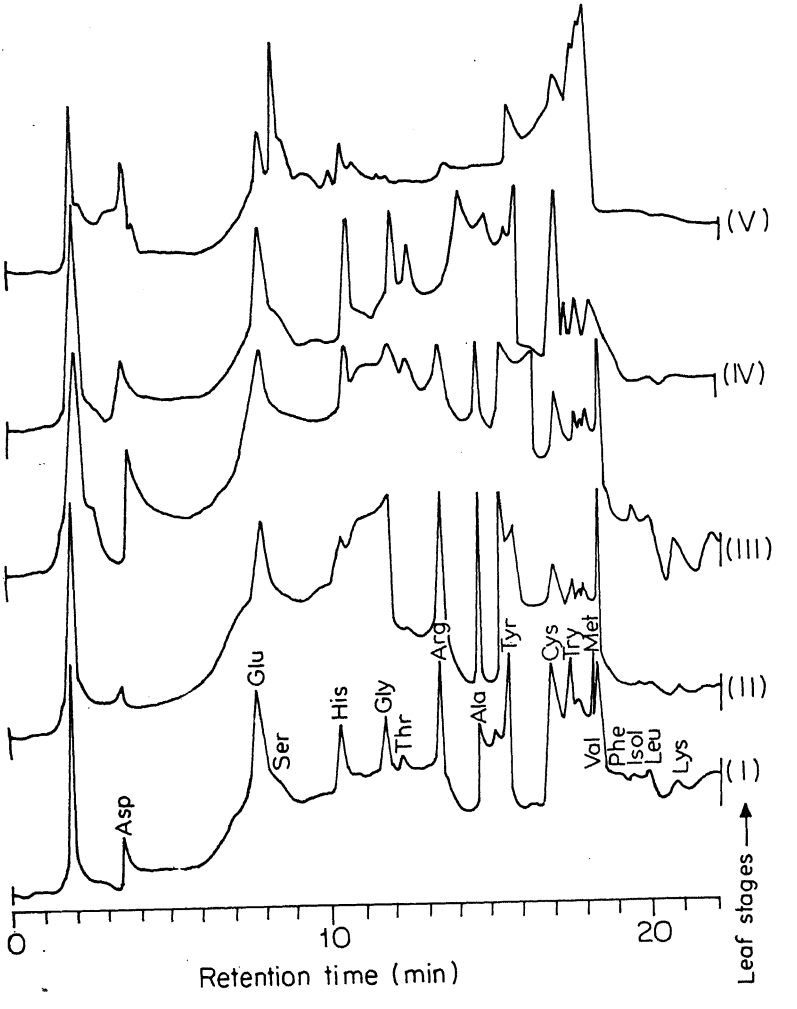

Figure 3. HPLC chromatograms of amino acids in different leaf stages of *T. catappa*.

4. Discussion

Natural infestation of leaves of *T. catappa* by *R. cruentatus* indicated a specific pattern in relation to both time and space, infestation occurring during a specific period i.e. March–September with the peak population of adults during the months of August/September. Infestation being specific to the abaxial side of the comparatively young, completely opened, fresh maturing leaves (stage III and continuing to stages IV and V), selective exploitation for feeding site and subsequent reproduction were very evident. The patterns of infestation, feeding and reproduction as well as the population build-up appear to be in close correlation with the initial leaf age and its subsequent stages indicating the influence of chemical changes in the host leaves. The availability of a substrate with an optimal mixture of suitable nutrients as well as feeding stimulants offered by any part of the leaf at any time cause such specific

Table 1. Amino acid profile in different stages of *T. catappa* leaves.

Amino acid	Retention time (min)	Area percentage* Leaf stages I	II	III	IV	V
Asparagine	3·50	02·99	00·346	13·849	02·240	00·847
Glutamine	7·77	12·55	4·704	14·742	3·619	06·944
Serine	8·24	ND	ND	ND	ND	ND
Histidine	10·30	07·57	03·357	03·458	00·392	05·729
Glycine	11·65	ND	05·654	04·840	05·175	05·706
Threonine	12·20	ND	04·875	01·486	ND	02·377
Arginine	13·35	ND	10·795	26·466	30·462	09·205
Alanine	14·50	ND	04·091	00·376	08·736	03·854
Tyrosine	15·25	05·50	03·611	08·130	09·216	11·610
Cysteine	16·75	03·056	06·174	03·387	06·728	06·306
Tryptophan	17·40	02·715	03·875	01·465	02·667	05·920
Methionine	17·75	02·977	03·132	01·703	03·588	01·544
Valine	18·20	ND	23·596	11·946	16·984	18·585
Phenylalanine	19·10	03·799	ND	02·559	ND	03·614
Isoleucine	19·70	01·778	10·246	ND	ND	11·261
Leucine	20·45	00·293	11·013	00·889	03·038	04·339
Lysine	21·55	03·867	04·801	00·378	ND	ND

*Based on amino acid peak areas only.
ND, Not detectable.

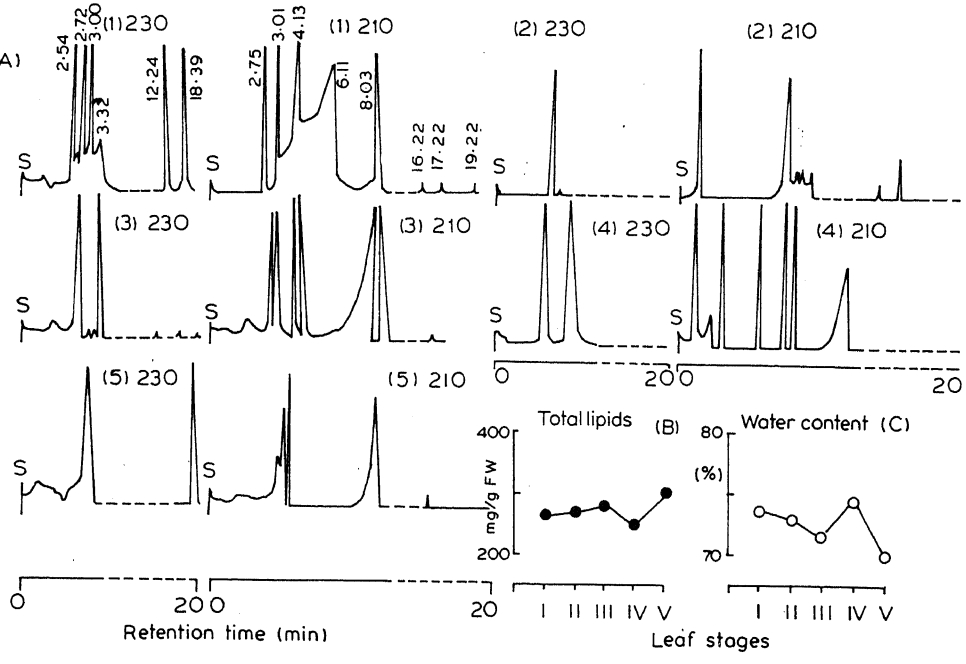

Figure 4. **A.** HPLC chromatograms of fatty acid methyl esters of different leaf stages of *T. catappa*. Numbers in circles indicate leaf stages. 210 and 230 indicate detection wavelengths. S, Start position. **B.** Total lipids in different leaf stages. **C.** Water content in different leaf stages.

Table 2. Qualitative profile of fatty acid methyl esters in different leaf stages of *T. catappa*.

Retention time (min)		Area percentage[a]				
		Leaf stages				
		I	II	III	IV	V
0·76		—	—	—	1·448[b]	—
1·20		—	9·803[b]	—	—	0·114[b]
2·50			—	—	1·204[b]	—
		0·589[c]			96·184[c]	
2·75		0·438[b]		—	—	—
		0·503[c]	99·44[c]			
3.00		76·206[b]	0·540[b]	1·396[b]	—	—
		4·961[c]		1·435[c]		
3·25			—	2·124[b]	—	4·823[b]
		−65·685[c]		0·789[c]		71·020[c]
4·00		—	—	0·490[b]	39·892[b]	—
				89·620[c]		
4·15		0·854[b]	—	76·071[b]	1·760[b]	—
5·5		—	44·922[b]	—	16·450[b]	—
6·0		5·184[b]	2·347[b]	—	—	—
6·25		—	2·430[b]	—	—	—
6·65		—	1·069[b]	—	—	—
8·50	Linolenic C18:3	8·606[b]	—	6·691[b]	33·351[b]	11·737[b]
				1·132[c]		
16·25	Oleic C18:1	0·606[b]	15·527[b]	0·393[b]	—	28·930[b]
17·25	Stearic	2·709[b]	15·385[b]	11·118[b]	—	3·607[b]
		25·253[c]		5·097[c]	—	28·930[c]
18·90		5·154[b]	—	—	—	—
		8·944[c]				

[a]Based on fatty acid methyl esters peak areas only.
[b]At 210 nm.
[c]At 230 nm.

infestation patterns. Alternatively it may also be due to the optimal substrate availability 'within a unit of actual feeding time' at specific circumstances sufficient to let the thrips live, grow in size and to lay eggs (Fennah 1963). The latter postulate may explain the infestation of the III, IV and V stage leaves due to the fact that the optimal concentrations of different nutrients are not available in all stages of leaf development.

Differential feeding requirements of insects and the diversified proportional composition of food chemicals among different plant species makes it a finely balanced problem for the insect to obtain an adequate intake of proteins and amino acids. This gets more complicated due to the variation in the leaf nitrogen content as well as water content in relation to seasonal changes. A comparative analysis of leaves of differing age of *T. catappa* in relation to infestation revealed an increasing protein concentration upto the III and IV stages, while the quantitative amino acid profiles showed a diametrically opposite pattern. However, the qualitative analysis and quantitation of individual amino acids showed that higher values were recorded either in stage III or IV, which coincides with the infestation and population build-up of *R. cruentatus*. A similar result was obtained, in the case of the sap sucking

insects, where the bulk of the population becoming adults coincided with the higher levels of amino acids in the infested leaves, and higher methionine levels correlated well with alate production. This could be true in the case of *R. cruentatus* since higher methionine levels were recorded in stage IV. In the senescing yellow leaves the infestation is absent, and that could be correlated with low nitrogen levels due to remobilization of unspent nitrogen by the plant.

Varying concentrations of nutrients in different feeding sites of a plant result in many insects feeding on different parts of their host plants, an aspect correlated with the sites of maximal nitrogen availability (Feeny 1970; Parry 1974; McNeill 1973). This could explain the infestation by *R. cruentatus* near the venal regions in the III leaves and on the laminar sides in the IV and V stages. In seasonal habitats synchronous flushing is a fundamental defence mechanism telescoping the essential transport of nitrogen into the shortest possible period (McNeill and Southwood 1978), and this high degree of synchrony may be disadvantageous to *T. catappa* since this thrips species seems to have been able to synchronise with the timing of the leaf flush and nitrogen content.

Qualitative profiles of fatty acid methyl esters in different stages of leaf development clearly revealed higher numbers in the early stages and from stage III onwards where infestation was initiated, as many as 7 fatty acid methyl esters were recorded, the important ones being the polyunsaturated C18:3 linolenic acid, the monoenoic C18:1 oleic acid and C18:0 stearic acid. Linolenic acid was present in higher concentrations in stage IV, while high stearic and oleic acid contents were noted in stage V, well correlating with the development of larvae, eclosion and at finality the egg deposition by females. It is well known that insects depend on dietary fatty acids in higher concentrations for the aforesaid and there is unequivocal evidence that some fatty acids might be more than a facultatively utilizable energy source since the di- and trienoic polyunsaturated fatty acids appear necessary for larval growth, pupal eclosion and wing expansion in insects. Among several saturated monoenoic fatty acids, oleic (C18:1) was most effective for silkworms, while in the majority of the cases, mixtures of fatty acids were superior to any one alone (Dadd 1973). Hence, it is not surprising that *R. cruentatus* had a preference for the leaf stages III–V since they not only provide higher fat contents than the rest of the stages, but also a mixture of essential fatty acids including linolenic, oleic and stearic acids, for the larval growth, pupal eclosion and subsequent reproduction within the specified time of the duration of different instars.

Available information on the influence of secondary plant substances, especially phenolics, largely gives the impression that they are the main defence arsenals of the plants and once the insect succeeds in dealing with these compounds, it could successfully colonise and utilise the host, though it extends far beyond the host selection phenomenon. Quantitative estimations of the key enzymes of secondary metabolism namely the phenylalanine ammonia lyase, tyrosine ammonia lyase and peroxidase showed a correlatable influence on the levels of total phenols as well as *o*-dihydric phenols that are of importance in host selection and feeding as well as the selection of the feeding stages and site. Of considerable relevance is the selection by *R. cruentatus* of the leaf stages of *T. catappa* where decreasing levels of total and *o*-dihydric phenols were recorded. If it were a rule that plant phenolics restrict insect colonization, then it may not be surprising to note that *R. cruentatus* chose a stage of decreasing phenolic contents with decreasing enzyme levels thereby having limited

profiles of repellent/resistant compounds including quinones in the leaf tissue. A further analysis of terpenoids and tannins on one hand and the alkaloids and glucosinolates on the other, the former providing barriers and the latter being utilised by the insects, would reveal reasons for the host selection and site selection phenomena of *R. cruentatus* on differing leaves in terms of age.

Total carbohydrate levels showed lesser variation among different leaf stages indicating that total carbohydrates may not be a limiting factor for specificity and feeding site selection, though in combination with other nutrients it would have a limiting influence.

Variation in leaf water content was not marked among different leaf stages of *T. catappa* (figure 4c) although it is known to decline with increasing age (Scriber and Slansky 1981). Rapid decline in nitrogen and generally lower leaf water in trees alone as influencing factors for feeding by insects is more difficult to interpret due to other accompanying chemical changes. Hence, within plant variability in nutritional and allelochemic composition such as phenolics would form important factors influencing site selection, synchronized life cycle pattern in relation to leaf flush, feeding and reproduction of *R. cruentatus*. Scriber and Slansky's (1981) conclusion that it is apparent to identify the specific proximate factors associated with the food quality and quantity of plant nutrients and allelochemics change through time and in response to environment, the behavioural responses resulting from change in food quality, as well as the utilization of food by arthropods, involves dealing with a multitude of covarying biochemical and morphological mechanisms that interact as food is ingested might exemplify the complexities of *R. cruentatus—T. catappa* interactions.

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Effect of sublethal doses of dichlorvos and carbaryl on the nervous system of *Pericallia ricini* Fabr. (Lepidoptera: Arctidae)

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Abstract. Topical application and injection of various doses of dichlorvos and carbaryl to the fourth instar larvae of *Pericallia ricini* did not alter the normal differentiation of various centres of the brain during metamorphosis but certain changes were produced in the neurons and neurites. These changes included the thickening of the neurilemma, clumping of chromatin, disintegration of cells and swelling of nuclei. The neurilemma of treated larvae became wavy, remained as such till the early adult stage and then degenerated for a very short period. The optic lobe anlagen cells exhibited increased mitotic division.

Keywords. *Pericallia ricini*; sublethal doses of insecticides; histopathology of nervous system.

1. Introduction

One of the main factors which may account for the lack of information on the action of the sublethal doses of insecticides is the production of insecticide resistant individuals. Thus most of the earlier workers paid their attention to study mode of action and histopathological effects of various insecticides at lethal levels (Richards 1941, 1943; Wigglesworth 1941; Hartzell and Scudder 1942; Salkeld 1951). Very recently Shaheen and Singh (1986) gave an account of the effects of sublethal doses of two insecticides on the nervous system of *Diacrisia obliqua*. They found a number of histopathological changes in the neurons and neurites by the application of very low doses of these insecticides, however, they did not find any alteration in the general differentiation of the brain centres during metamorphosis which was an interesting phenomenon. These observations fascinated us to gather some more information in other insects.

2. Materials and methods

Larvae of *Pericallia ricini* Fabr. were collected and reared in the laboratory. Fourth instar larvae were selected from the stock and all the experiments were performed at this stage. Four sets of 50 larvae each were placed separately in petridishes. Two insecticides viz dichlorvos [0, 0-dimethyl 2, 2-dichlorovinyl phosphate (DDVP)] and carbaryl [1-naphthyl-N-methyl carbamate (sevin)] were used for direct injection and topical applications. These were diluted with acetone to make 0·2, 0·4, 0·6, 1·0, 1·4, 1·6, 2·0, 4·0, 6·0 and 8·0 μg. Insects of the first set were treated with these insecticides topically on the dorsal region of the abdomen with a microapplicator. In the abdomens of the larvae of the second set, above mentioned doses of these insecticides were injected. The third set was treated with the same amount of acetone only and the fourth set was left untreated. Two to three insects of each set were dissected

at an interval of 2 days till the emergence of adults and the entire nervous system was fixed in aqueous Bouin's fixative for 18–24 h. The adults obtained from these treated larvae are termed here after as t-adults. The sections were cut at 6–8 μm thickness and staining was done with haematoxylin-eosin and Heidenhains Azan stains.

3. Observations

3.1 *Changes in the neurilemma*

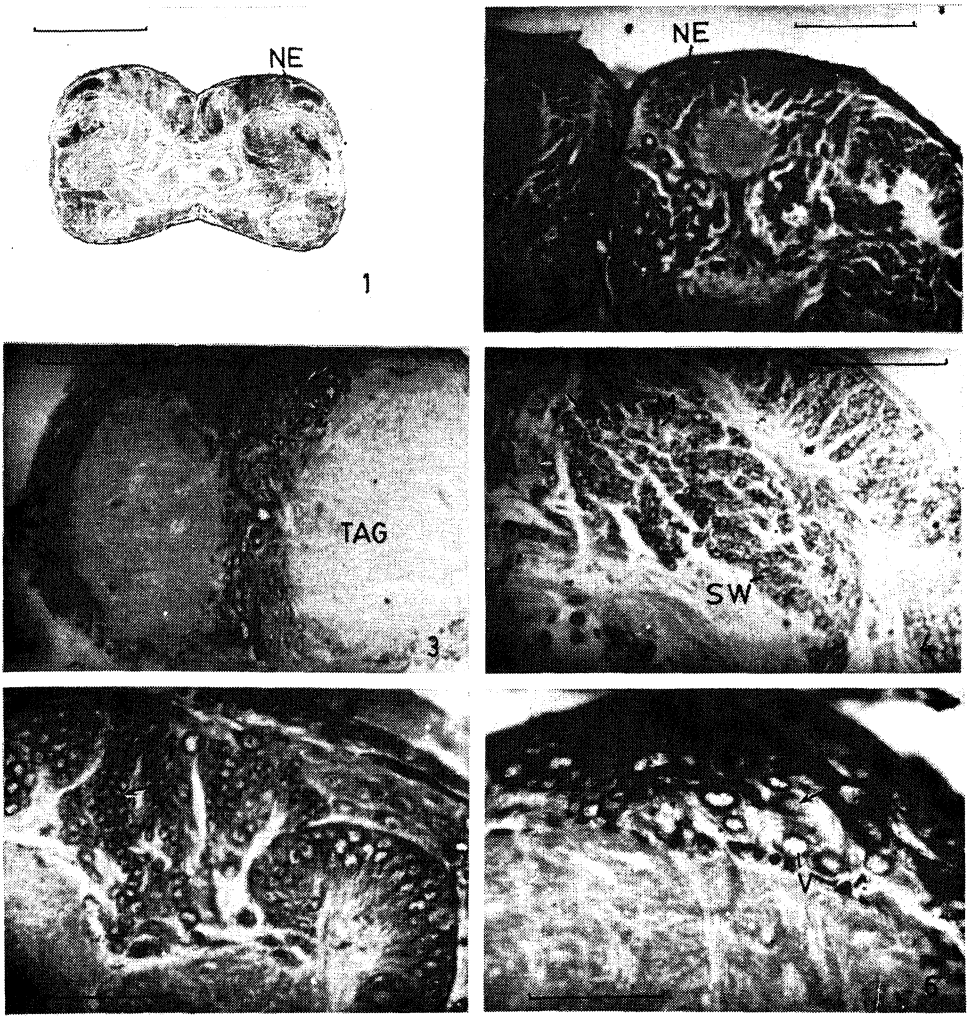
The normal thickness of the neurilemma of the brain and ganglia of *P. ricini* larva was about 0·75–1·0 μm (figure 1). After 4–12 days of topical application of 0·2, 0·4, 1·0 and 1·6 μg of sevin and 0·6, 1·4 and 1·6 μg of DDVP its thickness increased (figure 2). It was about 2·7–3·0 μm and 2·25 μm in the brain and nerve cord respectively. It also became wavy in the larval period. This type of wavy neurilemma appears during the mid pupal stage, prior to its degeneration in untreated insects. In the adults produced by the insecticide treated larvae (t-adults) the neurilemma of the thoracoabdominal ganglionic mass showed greater degree of wavy appearance. It also increased in thickness by the treatment of larvae with 1·6 μg of sevin. This wavy nature of the neurilemma appeared 4 days after the treatments of the larvae and did not degenerate during pupal period (figure 3).

3.2 *Changes in the cortical and neuropilar regions*

Various histopathological changes were produced by the topical applications and direct injection of the insecticides. These changes were more or less similar in both the methods except that the direct injection of insecticides produced these changes more rapidly. The 4th instar larvae of *P. ricini* treated with various doses of two insecticides and the two methods exhibited differentiation of all the centres of the brain in 5th instar larvae, pupae and adults like normal insects, however, changes were produced in the structure of the nerve cells and their neurites.

The central nervous system of the t-adults produced by the treatments of 4th instar larvae (8·0 μg of DDVP and sevin) showed slight changes in its histology. The neurilemma covering the thoracoabdominal ganglionic mass and abdominal ganglia became more wavy. The brain of t-adults showed swollen nuclei with an increase in the number of degenerating cells. The connective tissue sheath which appears during the absence of the neurilemma, also showed pyknosis.

Application of 6·0 μg of DDVP to the 4th instar larvae, after 2–7 days of treatment, showed swelling in the neurons with slight degeneration of chromatin and cytoplasm in the brain. Distinct swelling of globuli cells was noted in the calyx with chromatin attached to the periphery of the nuclei. The neuropilar regions of the larval brain and adults ganglia showed disintegration and degeneration of the longitudinal fibres. The newly emerged t-adults showed degeneration of neurons and irregular spaces in the perineurium and in between the cortical and neuropilar region of the abdominal ganglia. Such histopathological changes were not met within the control insects. Increased intercellular spaces and degeneration of longitudinal fibre tracts were noted in the thoracoabdominal ganglionic mass. The t-adults produced by the treatments with 4·0 μg of DDVP did not show any marked change in the

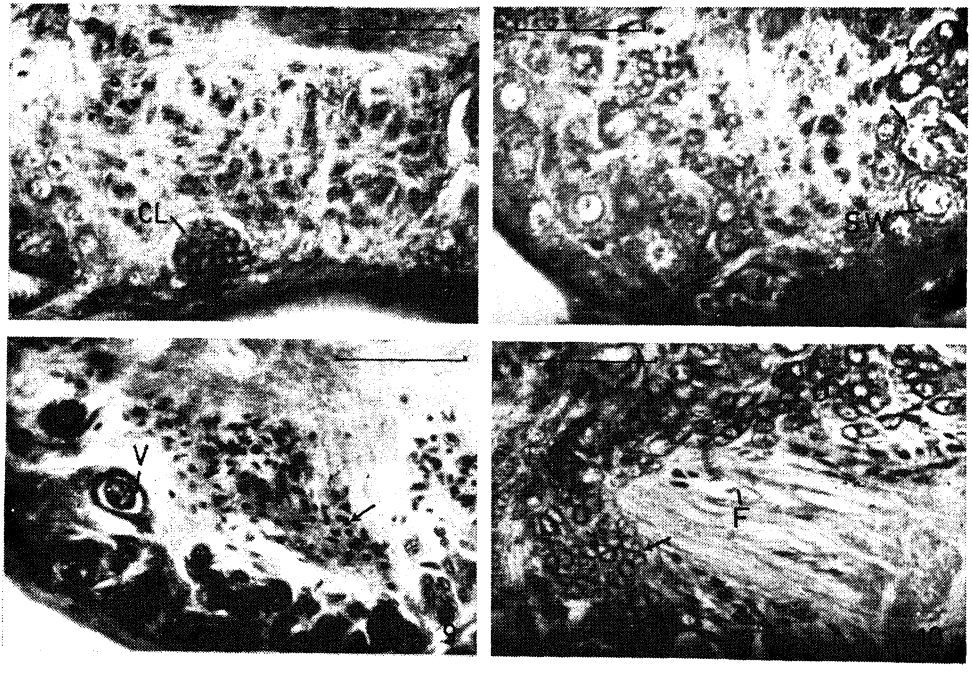


Figures 1–6. **1.** Frontal section (FS) of 5th instar larval brain of untreated *P. ricini* showing normal thickness of the neurilemma (NE). (scale bar—400 μm). **2.** FS of 5th instar larval brain showing an increase in thickness of the neurilemma (NE). Sevin, 0·2 μg. (scale bar—200 μm). **3.** FS of the thoracoabdominal ganglionic mass (TAG) of adult showing degeneration of the neurilemma and shrinkage of cells in the cortical region (arrow). Sevin, 1·6 μg. (scale bar—200 μm). **4.** FS of 5th instar larval brain showing mitic division (M), swollen nuclei (SW) and peripheral chromatin (arrow). Sevin, 1·6 μg. (scale bar—100 μm). **5.** FS of 5th instar larval brain showing peripheral accumulation of chromatin (arrow). Sevin, 2·0 μg. (scale bar—100 μm). **6.** FS of thoracoabdominal ganglionic mass of adult showing degeneration of neurons (arrow) and appearance of vacuoles (V) in nuclei. DDVP, 1·6 μg (scale bar—100 μm).

cellular integrity of the brain. In the ganglia, like 2·0 μg of sevin, the disappearance of chromatin with more marked degeneration of cells and production of dark stained particles around the neuropile was noted. No change was noted in the differentiation of optic centres and central complex. However, an enormous increase of the mitotic division of the optic lobe anlagen cells was observed (figure 4).

Treatment with 2·0 μg of DDVP and sevin on the larvae of *P. ricini* showed the clumping of chromatin which got attached to the nuclear membrane (figure 5). Gradual disintegration of cytoplasm and appearance of intercellular spaces were also noted. No marked changes were noted in the prepupal brain with sevin. With 1·6 μg of DDVP degeneration and shrinkage of cells and partial atrophy of fibres occurred in the adult's central nervous system. The ganglia showed enlargement of cells with enormous vacuolation of the nuclei (figure 6). With 1·0 μg of DDVP, pyknosis and breakage of the cell wall and thickening of the larval neurilemma was noted.

Very little cellular disintegration and changes in the nuclei were noted with 0·2, 0·4 and 0·6 μg of sevin and DDVP. The abdominal ganglia of treated larvae (0·2 μg of sevin) showed the appearance of clusters of small neurons placed in the peripheral part of the cortex very near to the thickened neurilemma (figure 7). 0·4 μg of sevin produced dark stained particles in the larval ganglia abundantly (figure 9) whereas 0·2 μg caused chromatin clumping, swelling of nuclei with intracytoplasmic vacuoles and cell degeneration in the larval ganglia (figure 8). Adults produced by the treatment with 0·8 μg of DDVP to the larvae showed degeneration of neurites and the peripheral accumulation of chromatin in the globuli cells of the calyces of the mushroom bodies (figure 10).



Figures 7–10. **7.** FS of 5th instar larval abdominal ganglion showing clusters of small cells (CL). Sevin, 0·2 μg. (scale bar—100 μm). **8.** FS of 5th instar larval abdominal ganglion showing swollen nuclei (SW) and degeneration of cells (arrow). Sevin, 0·2 μg. (scale bar—100 μm). **9.** FS of 5th instar larval thoracic ganglion showing dark particles (arrow) and vacuoles (V) in the cytoplasm of motor neurons. Sevin, 0·4 μg. (scale bar—100 μm). **10.** FS of adult's brain (t-adult) showing degeneration of fibres (F) and peripheral accumulation of chromatin. DDVP, 0·8 μg. (scale bar—100 μm).

4. Discussion

Wilcoxon and Hartzell (1933) observed vacuolation and trigrolysis of the Nisle granules and tissue disintegration in the brain and ganglia of the ventral nerve cord and to some extent in the connectives also in the larvae of *Tenebrio molitor* after treatment with pyrethrum. Klinger (1936) in the larvae of gypsy moth, *Porthetria dispar*, observed that a high dose of pyrethrum, 24 h after application, caused extensive damage to the nerve cells of the abdominal ganglia, especially towards the periphery. On the other hand, a number of well known insecticides do not produce any visible histopathological changes in insects. Kruger (1931) and Hartzell (1934) failed to find any effect on the central nervous system by applying rotenone. Thus it is evident that the mode of action and effects of different insecticides is not uniform in all the insects.

It is also known that the neurilemma of the insect central nervous system plays a role in the transport of nutrients in the nervous system (see Chandra and Singh 1982), assists to control the movements of ions into and out of the nervous system (Hoyle 1953; Pichon *et al* 1972; Lane 1972; Schofield and Treherne 1978) and performs an osmoregulatory function (Lorentè de No 1952; Shanes 1953). Like nutrients, the insecticides also might be entering through the neurilemma, perineurium and ultimately reaching to the glial cells, neurons and axons. The presence of bound lipid nerve sheath and their probable relation to the penetration of neurotoxic insecticides is of major interest in any study of mode of action of these toxins. Most of the earlier workers reported the destruction of this sheath by the insecticidal action thus facilitating the insecticides entry, but we do not find this type of destruction after the treatments with sublethal doses of dichlorvos and carbaryl on *P. ricini*. Thus there may be two possibilities for the entrance of these chemicals inside the cortex of the central nervous system of the insects—(i) either the entire lipid sheath, covering the nervous system degenerates or (ii) the insecticides diffuse through the tissue barriers (connective tissue sheath, neurilemma, perineurium and cortex). The former type of insecticide entry is reported by Klinger (1936), Richards (1941, in mosquito larva) and Wigglesworth (1941). Shaheen and Singh (1986) in *Diacrisia obliqua* and in the present study found cellular destruction in treated insects but did not include the destruction of the nerve sheath. This may be possible that either lower doses of these chemicals do not cause sheath destruction or that this change is insecticide specific.

Insecticides induce the division of cells. An enormous cell death due to insecticidal actions is most probably compensated by this increased cell division. The increase in the mitotic figures in treated insects might be due to this reason. The larval neural lamella is retained for a maximum period. In normal development it is degenerated in the early pupal period but in the treated insects it is retained till the early adult stage. It indicates that the treated insects try to retain the neural lamella as far as possible to avoid the entry of the chemical(s) present in the haemolymph. Other morphogenetic changes and differentiation of various nervous centres are not affected by the insecticidal treatment because the metamorphic changes in the central complex, optic ganglia and deutocerebrum etc take place normally and timely.

Treatments with dichlorvos and carbaryl produce vacuoles in the brain and ganglia. Clumping of chromatin, disintegration of cellular components and neurites, swelling and deformities in the nerve cells have also been noted. Particle formation is

also noted (Richards and Cutkomp 1945; Shaheen and Singh 1986). Appearance of vacuoles in the perineurium is also noted which may be due to the neuronal death caused by insecticidal action.

On the basis of the present study we have come to the following conclusions: (i) Treatments with dichlorvos and carbaryl do not interfere with the normal development, morphogenetic changes and differentiation of various centres of the brain and ganglia, but cellular and fibre disintegration of various degrees occurs, (ii) that treated insects try to retain the larval neural lamella for a maximum period and (iii) that insecticides probably induce cell division.

Acknowledgements

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Effect of starvation on food utilization in the freshwater snail *Pila globosa* (Swainson)

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Abstract. Increase in food supply produced elevated values of several parameters in energy budget of both normal and starved individuals ($P<0{\cdot}001$). Starvation accelerated the rates of feeding, absorption and metabolism (3 times) and conversion (1½ times; $P<0{\cdot}001$). *Pila globosa* starved for a month lost 79·5 mg dry weight/g live weight and the recovery periods to attain the original body weight were estimated as 66, 72, 88, 32, 28 and 23 days between 8 and 20% rations.

Keywords. *Pila globosa*; starvation; feeding; conversion.

1. Introduction

This paper is in continuation of a series on food utilization in freshwater snails exposed to different rations (Haniffa and Pandian 1974; Vivekanandan *et al* 1974; Haniffa 1982), food combinations (Haniffa *et al* 1984; Haniffa and Sethuramalingam 1985), crowding and water level (Haniffa 1980) and aestivation (Haniffa M A, unpublished results). Starvation is by definition a condition in which the animal's ration is not sufficient to balance its basal demand for energy. Under this condition the reserve food energy within the body is utilized in order to make good the difference between ration and respiratory loss (see Bayne 1973). Studies on the effect of starvation on metabolism of molluscs are well documented (Martin and Goddard 1966; Emerson 1967; Emerson and Duerr 1967; Stickle and Duerr 1970; Uma Devi *et al* 1986), but that on food utilization is meagre (Haniffa and Mullainathan 1982). The present work examines the effects of starvation on mortality, weight loss and food utilization in a freshwater snail *Pila globosa.*

2. Material and methods

Active individuals of *P. globosa* were collected from the pond Idumban (Palni, Tamil Nadu) and acclimated to laboratory conditions. About 150 snails of 12 ± 2 g live weight were recruited after 12 h of exposure to air (Haniffa 1978a) and introduced in groups of 25, into 6 glass aquaria (25 l capacity). Water was changed once in two days and the snails were starved for a period of two months; every week, mortality rates and weight losses were recorded. Fifty snails, starved for a month were divided into 10 groups, each containing 5 individuals and kept in troughs of 5 l capacity. Experimental snails were fed on *Chara fragilis* at chosen feeding levels ranging from

2–20% of their respective live weights. Everyday a control sample of 5 g food plant was dried at 85°C and weighed to measure its water content. Fifteen such starved snails were dried and weighed to determine the initial dry weight. Unconsumed plant was removed daily, dried and weighed to estimate the amount of food consumed. Faecal pellets were collected daily by filtering the water from the entire trough through a fine sieve of 160 μm. This experimental setup was maintained for a period of one month.

The scheme of energy budget followed here is a slightly modified IBP formula (Petrusewicz and Macfadyen 1970).

$$C\text{ (consumption)} = P\text{ (growth)} + R\text{ (metabolism)} + F\text{ (faeces)}.$$

The sacrifice method (Maynard and Loosli 1962) was followed to estimate the initial and final dry weight (inclusive of shell) of the test snails. Following the same procedure energy budget was estimated for normal *P. globosa* and a comparative analysis was made between starved and normal snails.

3. Results and discussion

The data presented in table 1 show the mortality rate and weight loss of one month starved *P. globosa*. Survival was 99·6% for one week-starved individuals and it decreased to 70·6% for the 7 week-starved snails. Total mortality during the tenure increased from 0·4% in the first week to 29·4% in the last week and mean mortality estimated after one month starvation was 10·5%. Following the least mean square method, the slope was calculated as 1·032 ($Y = 1{\cdot}032x + 0{\cdot}072$). Analysis of correlation coefficient confirmed that increase in the period of starvation resulted in heavy mortality ($P < 0{\cdot}01$). As the test individuals were forced to starve, it is possible to suggest that the absence of food energy and the utilization of body reserve during the course of starvation resulted in mortality (Uma Devi *et al* 1986). Weight loss via metabolism (shell and body tissue) amounted to 2·65 mg dry weight for one month

Table 1. Effect of starvation on survival and weight loss of the freshwater snail *P. globosa*.

Period of starvation (weeks)	Survival (%)	Mortality		Weight loss (mg dry/g live snail)		
		Week (%)	Total (%)	Shell	Flesh	Total
1	99·6	0·4	0·4	1·20	3·01	4·21
2	97·8	1·8	2·2	0·74	3·21	2·96
3	93·9	3·9	6·1	0·62	1·70	2·32
4	89·2	4·7	10·8	0·39	0·71	1·10
5	83·3	5·9	16·7	0·33	0·59	0·92
6	76·9	6·4	23·1	0·23	0·54	0·77
7	70·6	6·3	29·4	0·32	0·006	0·33
		Correlation coefficient				
	$r - 0{\cdot}987$	0·845	0·987	0·899	−0·676	−0·956
	$P < 0{\cdot}001$	<0·01	<0·001	<0·001	<0·001	<0·001

$P < 0{\cdot}01$, Significant; $P < 0{\cdot}001$, Highly significant.

starved *P. globosa* and this accounted for 0·74 mg loss of shell weight/g live snail/day i.e. those snails which survived one month starvation, exhibited 25% decrease in shell weight. Increase in starvation period resulted in a loss of shell weight from 1·2 mg in the first week to 0·32 mg in the 7th week ($P < 0{\cdot}001$). According to Haniffa (1978b), shell weight of *P. globosa* which successfully survived 5 months of aestivation exhibited 5·25% decrease in total shell weight. *P. globosa* is capable of aestivating for a period of 5 years (Hyman 1967) but starvation is not possible beyond 90 days. Hence the weight loss via metabolism during starvation is comparatively more. Working on the mortality rate of *Lymnaea stagnalis*, Jose *et al* (1968) reported that pond snails can survive a starvation period of 6 weeks by utilizing the reserve food energy. Uma Devi *et al* (1986) reported that the tropical intertidal gastropod *Morula granulata* can be subjected to prolonged starvation of 70 days.

Increase in food supply/day enhanced the energy budget of *P. globosa*. Feeding rate of normal *P. globosa* increased from 2·2 mg/g/day at 2% to 18·4 mg/g/day at 20% ration level (figure 1). Corresponding increases in the rates of absorption and metabolism were from 1·7 – 13·7 mg/g/day and 4·2 – 11·4 mg/g/day. The gain in body substance increased from 0·5 mg at 10% to 2·2 mg/g/day at the maximum ration level of 20%. Feeding rates estimated for one month starved *P. globosa* at different ration levels ranged from 5·1 to 53·3 mg/g/day (figure 1). Starvation caused about 2 times increase in food consumption; it might have stimulated the nerve centres controlling the appetite and in response to this the snail consumed more food.

Both normal and starved *P. globosa* showed weight losses at lower rations (2–8%). For feeding rates of 12·1 and 20·9 mg/g/day, a gain in weight of 0·5 and 1·2 mg/g/day was observed in normal and starved snails respectively. Maximum growth rate was

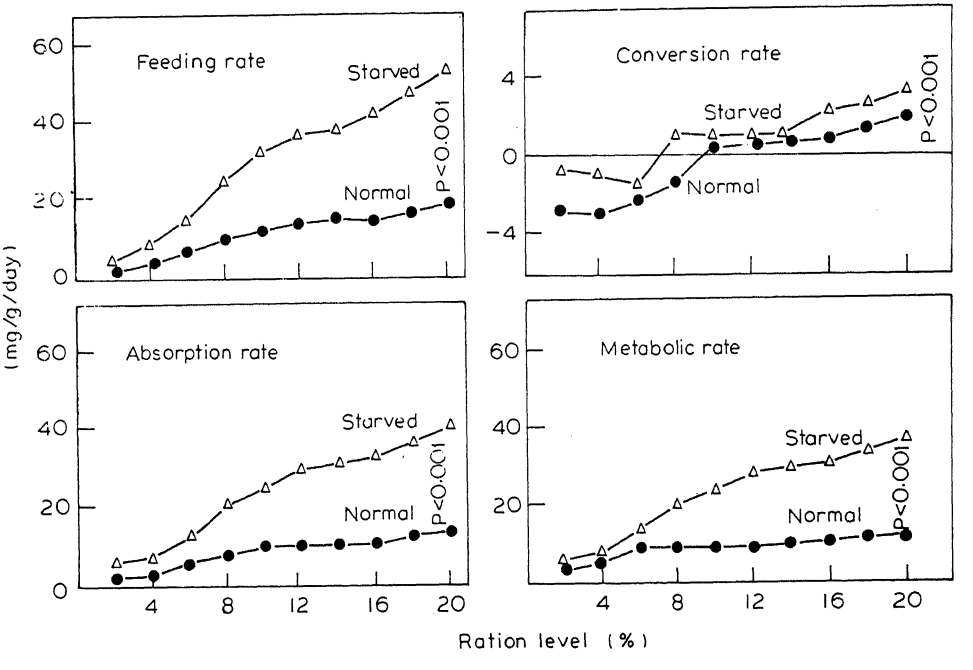

Figure 1. Effects of starvation on the rates of physiological energetics of *P. globosa*.

2·2 mg/g/day in normal snails and it increased to 3·4 mg/g/day for the starved *P. globosa* (figure 1). Haniffa and Mullainathan (1982) have also reported enhancement of conversion rate in the freshwater snail *Viviparus variatus* as a function of starvation.

Vivekanandan *et al* (1974) reported 14·7 gcal/g/day as the minimum energy cost of maintenance of *P. globosa* exposed to starvation and 67·4 gcal/g/day as the maximum energy cost of living for an active *P. globosa* fed *ad libitum* on *Ceratophyllum demersum* at 28°C. Considering the calorific content of *P. globosa* as 3682 ± 444 g cal/g dry weight (Haniffa 1978b), the energy lost through metabolism in one month starved *P. globosa* in the present investigation amounted to 9·8 gcal/g/day. When compared with normal snail, the metabolic level of one month starved *P. globosa* was lowered by 1/7th. The metabolic level of the aestivating snail was lowered by 1/18th of that of the normal snail (Haniffa M A, unpublished results). Similar observations on the capacity of the snail to lower its metabolic level have also been reported in *P. virens* (Meenakshi 1964), *P. ovata* (Visser 1965) and *Morula granulata* (Uma Devi *et al* 1986).

Increase in ration level caused rapid decrease in metabolism in normal snails but the decrease was slow in starved *P. globosa* (table 2). Starved snails also showed higher metabolic cost of 69–79% of consumption or 92–97% of absorption at higher rations (12–20%) than normal snails (62–71% of consumption or 84–93% of absorption). Hence it is possible to suggest that higher metabolic cost may be responsible for poor conversion efficiency in starved snails.

Increase in food supply enhanced the conversion efficiency from 5·6–12·1% in normal and 5·7–8·4% in starved *P. globosa*. Starvation elevated the values of conversion rate though the conversion efficiency actually showed decreases (table 2). Net conversion efficiency in the present investigation ranged from 5·6–13·0 and from 3·0–8·4% for normal and starved snails respectively. Increase in ration level caused a decrease in absorption efficiency from 83–74% and from 93–76% in normal and starved *P. globosa* (table 2) respectively (Vivekanandan *et al* 1974; Haniffa 1982). Absorption efficiency values reported for gastropods range from 73–88% for *P. globosa* (Haniffa 1982), 57–89% for *Littorina littorea* (Grahame 1973), 73–88% for

Table 2. Effect of ration level on efficiencies of absorption and conversion and recovery period to attain original weight by starved *P. globosa*.

Ration level (%)	Absorption efficiency (%)		Conversion efficiency (%)		Total loss of body weight (mg dry/g live snail)	Recovery period (days)
	Normal	Starved	Normal	Starved		
2	83·4	92·7	—	—	79·5	—
4	82·6	84·6	—	—	79·5	—
6	84·4	80·7	—	—	79·5	—
8	80·6	82·6	—	5·7	79·5	66
10	78·8	78·7	—	4·5	79·5	72
12	76·7	81·7	5·6	3·7	79·5	72
14	75·2	79·8	7·4	3·0	79·5	88
16	73·6	78·4	7·7	7·5	79·5	32
18	74·4	77·1	13·0	7·5	79·5	28
20	74·2	75·6	12·1	8·4	79·5	23

Gyraulus convexiusculus (Vivekanandan and Pandian 1976) and 64–80% for *V. variatus* (Haniffa and Mullainathan 1982).

Elevations in the rates of feeding, absorption, metabolism and conversion as a function of ration level in normal and starved *P. globosa* were statistically significant ($P < 0{\cdot}001$). Starvation accelerated the energy budget of *P. globosa* and Student's *t* test confirmed that this elevation was statistically significant ($P < 0{\cdot}001$; figure 1). Regression equations were fitted by the method of least squares and the coefficients were used in analysis of variance (Zar 1974). It was confirmed that feeding and ration level significantly influenced the absorption efficiency ($P < 0{\cdot}05$ and $< 0{\cdot}01$) and conversion ($P < 0{\cdot}05$) but the difference in metabolic rate (as percentage of consumption or absorption) was not significant ($P > 0{\cdot}05$) as a function of feeding or ration level (table 1).

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Limnology of river Coum with special reference to sewage and heavy metal pollution

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Abstract. The physical, chemical and biological variables and pollution of river Coum with sewage and heavy metal were studied in 4 stations within Madras city for a period of one year from August 1982 to July 1983. The physical, chemical and biological parameters presented complex pattern of variations indicating that the biotic life of Coum river is severely affected by the excessive discharge of sewage effluents within the city limits which excessively eutrophicate the river water. The number of species and density of zooplankton are very low. Heavy metals such as iron, nickel, lead and zinc are recorded to be above permissible levels.

Keywords. Lotic limnology; sewage pollution; eutrophication; heavy metals.

1. Introduction

Information on lotic limnology is mainly restricted to the pioneer works of Ganapathi and Alikunhi (1950), Chacko and Ganapathi (1949, 1952). Iyengar and Venkataraman (1951), Sreenivasan and Sounderaj (1967) and Govindan and Sundaresan (1979) in South India. Relatively little is known about the sewage and heavy metal pollution of South Indian rivers except the study of physical, chemical, biological and microbiological aspects of the river Coum made by Narayanan (1980); the report of the effects of pollution on the algal flora of the Cauvery river by Paramasivam and Sreenivasan (1981) and the detailed work done by Ravichandran (1985) in the Buckingham Canal with special reference to sewage pollution. In the present study a survey of the sewage and heavy metal pollution of Coum river was made to know the effects of eutrophication and heavy metal pollution on zooplankton population.

2. Materials and methods

2.1 *Study area and study design*

River Coum which starts from Sattarai village in Chingleput district flows just 65 km, passes through the city of Madras (Lat 13° N; long 80° 15′ E) and opens into the Bay of Bengal near Chepauk (figure 1). Bangaru Channel taking off from Kesavaram Anicut across Koratalyar joins this river and overflow from a number of tanks fall into the river before it reaches Madras.

Four stations were chosen for the collection of water samples in the stretch of the river within the city. Station 1 was located at Arumbakkam; station 2 at Chetpet; station 3 was at Chepauk while station 4 was the estuary.

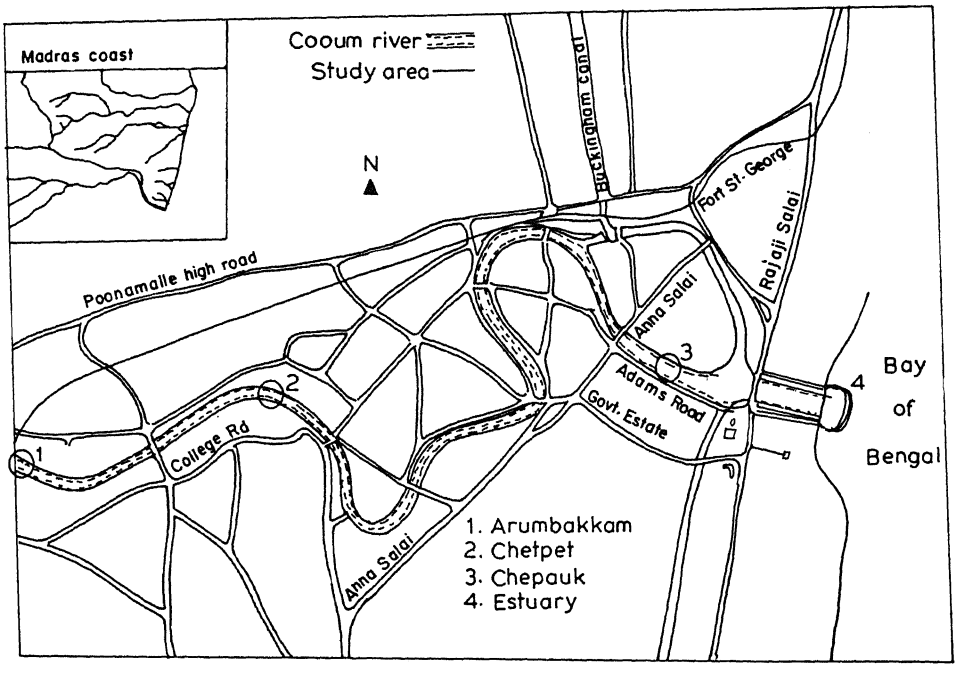


Figure 1. River Cooum – Sampling stations within Madras City.

2.2 *Sampling programme*

The 4 stations were sampled for a period of one year from August 1982 to July 1983. Once in a month samples were collected before 1000 h. A Vondorn sampler was used for the purpose. Two litres of water were collected in clean polythene bottles and transported immediately to the laboratory for analysis.

2.3 *Analytical methods*

The various methods used for the estimation of the variables are listed below:

(i) *Physico-chemical variables*:

Temperature	—	Recorded by a sensitive 0–50°C mercury thermometer.
pH	—	Recorded by Elico Model Li-10 pH meter.
Dissolved oxygen (fixed at the site itself)	—	Azide modification of Winkler method (APHA 1980).
Free carbon dioxide (fixed at the site itself)	—	Titrating against 0·045 N sodium carbonate with phenolphthalein as indicator (APHA 1980).
Alkalinity	—	Phenolphthalein and methyl orange alkalinity was estimated by titrating 100 ml samples against standard acid (Welch 1948).

Phosphate	—	Aqueous stannous chloride method after digestion with sulphuric acid-nitric acid reagent (APHA 1980).
Silicate	—	Silicomolybdic acid method (APHA 1980).
Nitrate	—	Phenol-di-sulphonic acid method (Mackereth 1957).
Sulphate	—	Turbidimetric method (APHA 1980)
Iron	—	Phenonthroline method (APHA 1980)
Lead	—	Dithizone method (APHA 1980)
Nickel	—	Dimethylglyoxime method (APHA 1980)
Zinc	—	Dithizone method (APHA 1980)

(ii) *Biological variable*: Zooplankton —Zooplankton were sampled by filtering 100 l of water through a plankton net of 32 μm mesh size, concentrating to 100 ml and preserving in 4% formal saline solution. Identification was done with standard keys (Ward and Whipple 1958) and plankters were enumerated with a sedgewick rafter counting cell.

3. Results and discussion

3.1 *Temperature*

The atmospheric and surface water temperature showed seasonal variations in all the 4 stations having an unimodal peak during summer. The surface water temperature closely followed the air temperature in all the 4 stations. This might be due to the shallowness and slow flow of the river; the temperature pattern of the surface waters following the atmospheric temperature is not an uncommon phenomenon and in most of the lotic systems this has been observed (Ganapathi 1956; Rai 1974a; Narayanan 1980; Ravichandran 1985). There was not much spatial difference between different stations.

3.2 *pH*

The pH value slightly increased during summer. The seasonal variations in pH of surface waters exhibited same pattern in different stations. The pH values fluctuated between 6·5–8·0. This narrow range of fluctuation in pH value throughout the year reflects the well buffered nature of the water. The range of pH values of this tropical river is identical with other than Indian rivers recorded by Subramanian (1979).

3.3 *Carbonate alkalinity*

The phenolphthalein alkalinity was observed only during summer months and not detected during most of the study period. The absence of carbonates might be due to the time of sampling (0900–1000 h). Similarly Sinada and Karim (1984a) and Ravichandran (1985) were not able to detect phenolphthalein alkalinity during these hours in blue and white Nile and Buckingham canal respectively.

3.4 *Bicarbonate alkalinity*

Slight spatial and annual variations were observed in bicarbonate alkalinity. Higher values in bicarbonate alkalinity were always recorded in station 4, which was grossly affected by sewage. Pollution by sewage and its subsequent decomposition has been quoted as possible causes for the increase in bicarbonate alkalinity of the waters of river Sabarmati (Venkateshwarlu and Jayanthi 1968) and the river Yamuna (Rai 1974a). The bicarbonate alkalinity values recorded in Cooum river were higher than that of Buckingham canal (Ravichandran 1985) showing the heavy impact of sewage pollution.

3.5 *Dissolved oxygen*

Dissolved oxygen content in the surface waters were very low fluctuating between nil values to 8 mg/l. Depletion of oxygen in all stations was mainly due to eutrophication and degradation of organic matter present in the sewage by the heterotrophic bacteria which completely take up all available dissolved oxygen (DO) (Hynes 1970; Welch 1980). Similar observations were made by Ravichandran (1985) in Buckingham canal which was also highly polluted by sewage.

3.6 *Free carbondioxide*

Fluctuation in the concentrations of free CO_2 in Cooum river was slightly linked with seasons, showing higher values during monsoon and lower values during summer. Occasional higher values of free CO_2 might be due to the release from the sediments during breakdown of organic matter. Similar sudden shoot up of free CO_2 was observed by Paramasivam and Sreenivasan (1981) in river Cauvery and Ravichandran (1985) in Buckingham canal.

3.7 *Phosphate*

Phosphate contents seemed to be high at stations 1 and 2. Phosphate varied from 0·1–7·94 mg/l. Maximum phosphate content was observed at station 2. The high values of total phosphate at stations 1 and 2 might be due to the sewage input and the excessive eutrophication of waters of the Cooum. High values of total phosphate were usually recorded in rivers/streams affected by domestic sewage (Shane *et al* 1971; Ravichandran 1985).

3.8 *Silicate*

Silicate values were high in all the stations except station 1. It ranges from minimum value of 0·2 mg/l to maximum value of 24·8 mg/l. Higher silica content was also recorded in various rivers of India by Subramanian (1979). The high silica content might be due to the decomposition of the diatoms as well as storm discharge (Sreenivasan 1970).

3.9 *Nitrate*

Nitrate nitrogen level varied between 0·77–11·76 mg/l. Higher values were recorded at stations 3 and 4. Concentration of nitrate nitrogen indicated clearly the impact of sewage discharge and eutrophication in Cooum. Similar higher values were also observed in Buckingham canal (Ravichandran 1985).

3.10 *Sulphate*

Sulphate in the surface layers of Cooum river showed a slight increase during monsoon in all the 4 stations. Sulphate values ranged from 80–408 mg/l. The values recorded were greater than those encountered in other Indian rivers (Subramanian 1979). Sulphate becomes the electron acceptor when the dissolved oxygen is completely depleted and used for the organic matter break down by bacteria and results in the production of H_2S which forms as bubbles at the surface (Welch 1980). High sulphate levels were also associated with sewage pollution. Similar observations were recorded by Govindan and Sundaresan (1979) and Ravichandran (1985) in tropical polluted lotic environments, Adyar and Buckingham canal respectively.

3.11 *Heavy metals*

Heavy metals when in normal concentration are essential components of biochemical functions but toxic when present in higher concentrations.

3.11a *Iron*: The iron content was found to be high in the first 2 stations. Maximum value of 900 mg/l was observed at station 2. The source of excess iron might be sewage. The concentration of iron was at the maximum during rainy season and it was below detection limits during January. Similar observations were recorded in Blue Nile (Sinada and Karim 1984a).

3.11b *Lead and nickel*: Maximum amount of lead (275 mg/l) was observed in station 1. Maximum nickel concentration of 1312·5 mg/l was also observed at station 1. The concentrations of lead and nickel were higher during rainy season. Irregular fluctuation of lead content in all the stations, especially at station 4, could be due to the fluctuations in the speed of river water (Ellaway *et al* 1980). The drastic fluctuation of nickel content was found to be greater in first 2 stations. Similar fluctuations in nickel content were made by Welch (1952) in polluted aquatic environments. The high values of nickel in this river might be due to the electroplating industries which are located on the banks of the river between stations 2 and 3. Further, the high values of nickel might be due to the failure of monsoon which resulted in evaporation of water and concentration of contents (Welch 1952). The high values might also be due to high pollution caused by sewage and organic matter (Lichtfuss and Gerbard 1981). It could also be due to the mixing of small amounts of enriched bottom waters with outflowing surface waters (Ellaway *et al* 1980).

3.11c *Zinc*: Maximum value of zinc (32·5 mg/l) was observed at station 1. Totally there was drastic fluctuations in the levels of zinc in all the 4 stations. The drastic

fluctuation of zinc content might be due to the slow speed of the Cooum river (Welch 1952). The content of the metal varies which could be due to high pollution caused by sewage entry and monsoon failure (Lichtfuss and Gerbard 1981).

3.12 *Zooplankton (figures 2–5)*

In the present study 14 species of zooplankton composed of protozoans, rotifers,

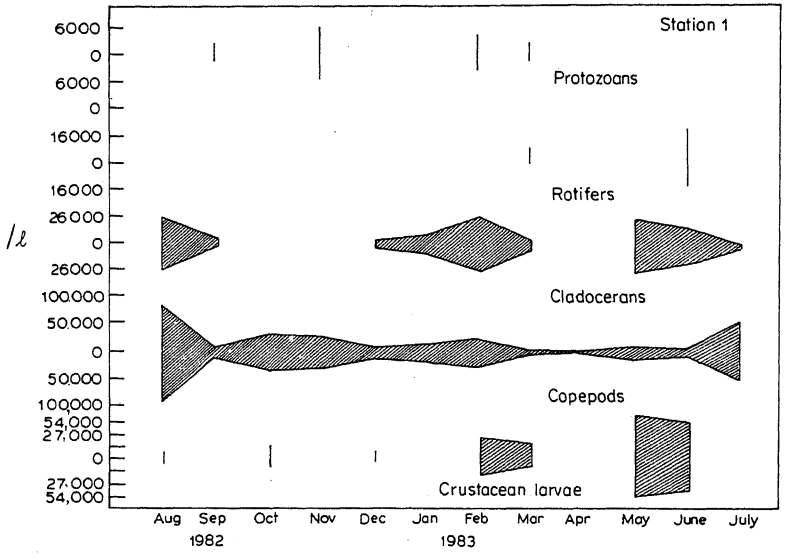
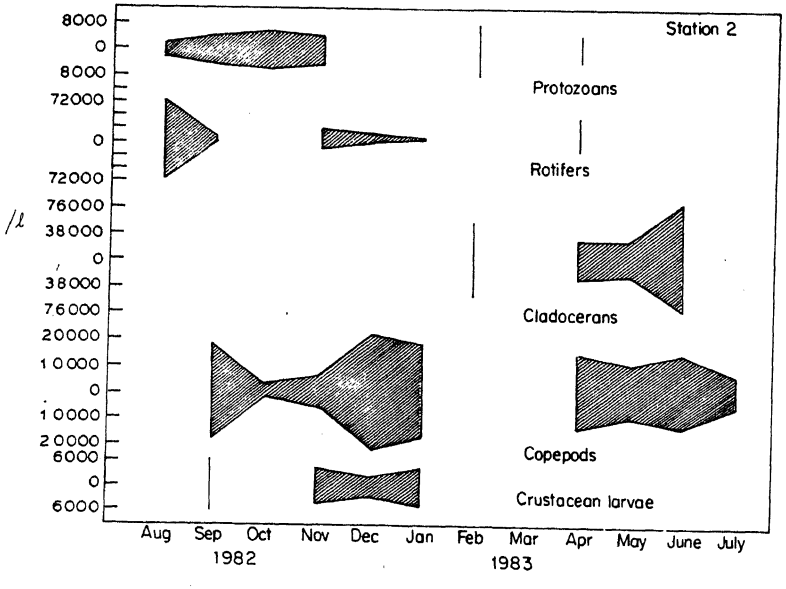


Figure 2. Zooplankton in river Cooum.



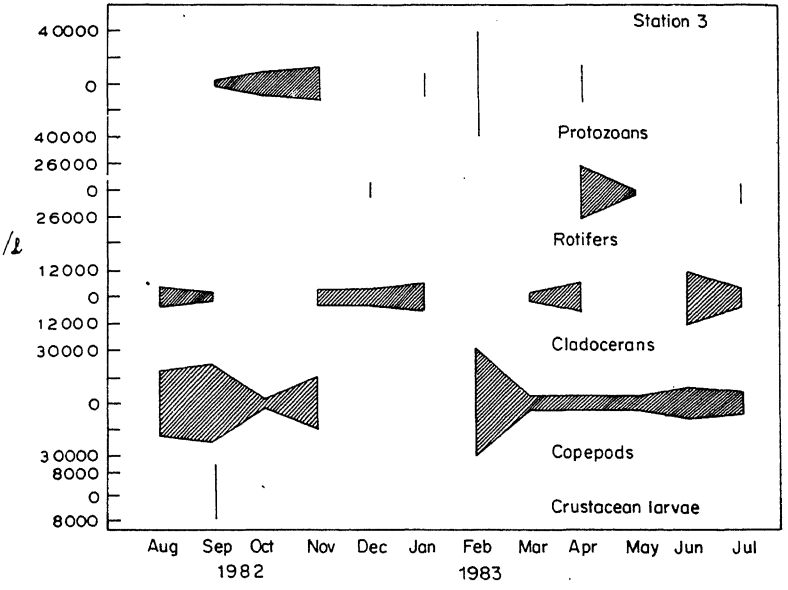


Figure 4. Zooplankton in river Coum.

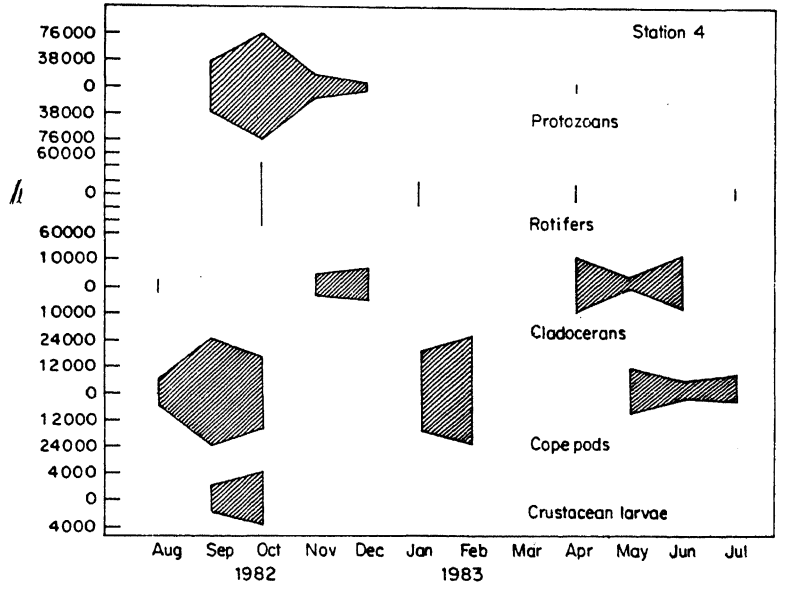


Figure 5. Zooplankton in river Coum.

copepods, crustacean larvae and cladocerans were observed. Cladocerans and copepods were present in large numbers. Rotifers occupy numerically the last place in order of abundance. Rotifers might have been replaced by copepods and cladocerans (Hynes 1970). The occurrence of crustacean larvae and copepods were patchy in different seasons in almost all the stations. Similar sporadic occurrence of cope-

pods have been recorded by Klimowicz (1973) who suggested that these organisms are present only when physical and chemical conditions were highly favourable. Cladocerans were recorded in all the stations. Off all the stations zooplanktons were abundant in station 1. A decrease in zooplankton was noticed in all the stations during rainy season. Similar observations of decrease in zooplankton during monsoon was seen in Buckingham canal (Ravichandran 1985). The impact of sewage pollution in Coum was clearly evident as the number of species recorded and density of zooplankton were very low. Identical recordings were made by Narayanan (1980) and Ravichandran (1985) in Coum and Buckingham canal respectively. The low levels of dissolved oxygen, higher levels of free CO_2 nutrients such as phosphate, sulphate and nitrate due to eutrophication and the toxic levels of heavy metal pollutants in Coum river waters might be the cause for its inability to support greater number and density of zooplankton populations.

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FOREWORD

Nutrition and reproduction are interrelated processes occurring in conjunction with behavioural and chemosensory facets of the biology of the insects and hence form essential parameters in our knowledge of host relationships, whether it be insect-plant interactions or parasite/predator-host interactions. While it is well known that insects do not differ considerably in their fundamental nutritional requirements, quantitative aspects combined with biochemical composition of the food in terms of the nutritional quality appears to have some bearing on the developmental biology of insects. Differences in the rates of larval development, survival, adult emergence, longevity and fecundity depend on the quality of the host tissues. As such an understanding of the nutritional indices in relation to the rates of ingestion, digestion, assimilation and conversion into insect tissues plays a useful role. Essential prerequisites for identification of specific factors influencing the efficiency of utilization relate to precise estimation of the biochemical factors involved. While many common nutrients are known to function as phagostimulants, gustatory chemical stimuli are crucial to a normal rate of feeding, especially in phytophagous and blood sucking insects.

Recent researches in insect nutrition studies involve an increased understanding of the metabolism of nutrients subsequent to their assimilation and ultimately directed towards their physiological functions. In other words, nutritional data are interpreted in terms of physiological functions using microanalytical procedures. Current interest is centred on lipid nutrient needs and it is well known that insects require dietary sterols for proper growth and utilization of sterols available in the plants which influence egg production. Essential fatty acids needed for metabolism combined with the identification of prostaglandins have opened up new vistas in insect nutritional studies. The role of sterols in moulting hormone physiology, in particular the newer patterns of phytosterol metabolism, has enabled a better understanding of insect-plant interrelationships integrating nutrition and reproduction. In haematophagous insects nutritional adequacy of the blood meal is closely associated with fecundity.

Bio-energetic studies have shown that protein-rich food is among the principal factors influencing fecundity. Feeding regimes also seem to influence the pattern of energy allocation for reproduction. The rate at which food is consumed appears also to be a basic factor on which the energy of the terminal larva, pupa or adult depends so that feeding rate tends to determine energy allocation for egg production. The degree of host preference also has a definite correlation with the age specific changes in the foliage of host plants coupled with the nutritive value of the food substances, which in turn influences the reproductive potential of the concerned species.

It is therefore of significance that the papers presented in this issue of the Proceedings, Animal Sciences in such areas as nutritional modulation of reproduction in phytophagous, haematophagous, predatory and parasitic insects, neuroendocrine control of insect nutrition and reproduction, as well as energy components involved will go a long way in furthering our knowledge in this very important field of entomological research.

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Nutritional modulation of reproduction in two phytophagous insect pests

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Abstract. In *Earias vittella* (F.) (a major noctuid pest of malvaceous crops) changes in the larval food quality (developing seeds, mesocarp, epicarp of okra fruit or the entire fruit; ovary of shoeflower) in either or both sexes led to pronounced disparities in the reproductive performance of the emerging moths. Highest breeding potential was observed when males and females obtained their nourishment, during postembryonic period, from developing seeds of okra of 0–8 days old and such nutrition must be made available for the caterpillars at least for the first 3 days of their lives to attain enhanced level of reproductive efficiency later as moths. During adult life, a carbohydrate nutrient was mandatory for these mated females to realize full fecundity which got tremendously boosted when the sugar was raffinose.

Appreciable increase in ovarian weight with marked improvement in oviposition occurred in *Tribolium castaneum* (Herbst) (a serious tenebrionid pest of cereals and other plant-derived stored commodities) when both larvae and adults ate whole flour instead of semolina, both enriched with yeast. This became further augmented when the flour ingested by these beetles during adult life was previously extracted in 100% ethanol and then reinforced with yeast. However, whole wheat flour or yeast alone given as food to such reared adults caused a sharp fall in the egg deposition of females. Number of eggs laid by mated females also differed considerably when their imaginal diet consisted of one of certain selected nutrients, oilseeds or spices—all supplemented with yeast. Egg hatchability was always 100%

Keywords. *Earias vittella*; *Tribolium castaneum*; larval; adult nutrition; reproductive potential.

1. Introduction

It is well known that reproduction in insects is influenced by a variety of external and internal factors and, amongst them, nutrition seems to be the most crucial single factor in affecting the total egg output in many ways in a majority of the species (Engelmann 1970). Its profound impact on the breeding potential of the adult can be a reflection of differences in the nourishment acquired by the concerned individual during its immature (larval/nymphal) and/or imaginal stage (Wigglesworth 1960; Johansson 1964; Adiyodi and Adiyodi 1974; Nayar 1977). Study of such effects of nutrition on the reproductive performance of the spotted bollworm, *Earias vittella* (F.) (a notorious noctuid pest of malvaceous crops like cotton and okra in the tropics) (Butani and Jotwani 1984) and the red flour beetle, *Tribolium castaneum* (Herbst) (a major tenebrionid cereal and other plant-derived stored product pest of cosmopolitan distribution (Sokoloff 1974; King and Dawson 1977) have been reported in diverse periodicals, during the past little over a decade by this author and his co-workers (Viswapremi and Krishna 1974b, 1975; Krishna *et al* 1977; Singh and Krishna 1980, 1983; Narayan Singh and Krishna 1980, 1981, 1983; Mani *et al* 1986). Findings from all these contributions are incorporated in this communication so as to constitute one comprehensive body of information.

2. Materials and methods

Laboratory stock cultures of *E. vittella* raised on tender seeds separated from 0–8 days old fruits of okra (*Abelmoschus esculentus* Moench) and of *T. castaneum* reared on whole wheat flour containing 5% (w/w) powdered yeast (BPC product, Alembic Chemical Works Co. Ltd., Baroda) supplied the required numbers of newborn larvae (< 24 h old) of these insects which served as the starting material for inclusion in the different investigations. Cultures of *E. vittella* were maintained at temperatures ranging between 25° and 28°C and relative humidity (RH) varying from 80–100% while those of *T. castaneum* were held within a thermal range spanning between 30° and 32°C and RH values fluctuating between 70% and 80%. All experiments described in this study and related to each of these two species were conducted at the same temperature and humidity ranges as were chosen for maintaining their cultures. These tests were adequately replicated and, wherever desirable, the data were subjected to appropriate statistical analysis (Paterson 1939; Snedecor 1961).

For the sake of convenience, details of the technical procedures involved in every experiment are separately given below for the two selected pest species.

2.1 *E. vittella*

In all the trials set up here, single-reared, freshly emerged adult moths of either sex were employed. Pairing, mating and individual oviposition tests were performed in muslin-covered glass containers (70 mm diameter; 90 mm height) each provided with a hanging glass capillary filled with 15% sucrose solution (unless otherwise stated) that formed the food of the adults. A small section of the epicarp portion of okra fruit serving as oviposition substrate was placed at the bottom of the container. In most cases, the daily monitoring of egg laying was limited to the first 4 days of oviposition (the period when mated females in their life time deposited most of their eggs) although in a few experiments it was continued further even up to the death of the females. The hatchability of the eggs was also ascertained.

2.1a *Effect of variation in larval nutrition*: Three types of experiments were designed to examine this issue. In the first type, the caterpillars were nourished right from the day of their birth on 0–8 days old whole fruit of okra or on its epicarp, mesocarp or seeds removed from it to yield male and female moths which were subsequently single-paired (both sexes belonging to the same food regimen) and the reproductive potential of the females assessed in relation to such nutritional differences. In the second type, the reproductive efficiency of moths associated with larvae allowed to feed only during their first 24 or 72 h of their lives on normal and fresh tender seeds taken out of okra fruit and then shifted to a diet consisting of normal and ripened seeds excised from 9–11 days old fruits was compared with that of counterpart adults when both sexes of reproducing pairs were reared all through on the latter victual or on petroleum ether-extracted tender seeds 72 h after they derived nourishment from normal and tender seeds.

Extraction of the seeds in petroleum ether was performed in a soxhlet extraction assembly. For this purpose, 100 tender seeds collected from fresh okra fruits (age as mentioned above) were first properly ground using a mortar and pestle in 25 ml of the solvent. Later, this crude seed extract was wholly transferred to the soxhlet assembly containing the same solvent and subjected to repetitive extractions within

the apparatus to ensure removal of all ether-soluble constituents. Such extracted seeds were subsequently taken out of the assembly and air-dried to eliminate completely the odour of ether present in them before offering them as diets to the caterpillars.

In the third type, okra seed diet-reared newly eclosed males or females were coupled with emerging members of the opposite sex grown on epicarp diet and the breeding capacity of females in these pairs determined. Pairs of individuals reared on the ovary component of shoeflower (*Hibiscus rosasinensis* L.) or on tender seeds or epicarp of okra fruit were also arranged to serve identical purposes.

2.1b *Effect of variation in adult nutrition*: Here male and female moths, developed on okra seed diet and paired as before for determination of the reproductive potential of this pest, were during their adult lives, allowed to imbibe only distilled water or solutions of one of 5 selected carbohydrates (raffinose, sucrose, fructose, galactose and glucose) provided through the hanging glass capillary and replenished daily. Although the concentration of all these sugars was maintained at 15%, the trisaccharide raffinose was tested at 5 different lower strengths (0·1, 0·25, 0·5. 1·0 and 5·0%) as well.

2.1c *Effect of variation in larval and adult nutrition*: Consideration of this aspect led to a comparison of reproductive competency of mated females included in pairs fed daily on fresh 15% solution of glucose and whose larval and pupal lives were raised on okra seeds with those associated with couples ingesting raffinose of similar concentration and developed from individuals reared on epicarp diet.

2.2 *T. castaneum*

This part of the investigation mainly related to determination of (i) ovarian weight and reproductive potential of the female reared and maintained on whole wheat flour or semolina supplemented with 5% yeast (w/w) and (ii) the influence of adult diet on the reproductive potential of the female.

For studying the first aspect, 10 larvae procured from the laboratory culture were collectively permitted to continue their postembryonic development up to pupal stage on whole wheat flour or on semolina enriched with yeast provided in sufficient amounts within glass containers (30 mm diameter; 105 mm height) covered at the top with muslin cloth. A male and a female pupa, distinguished on the basis of sexual dimorphic characters akin to those described for *T. confusum* (Chapman 1918), formed from such group-reared larvae were transferred as a single pair to a small muslin-capped glass vial (10 mm diameter; 50 mm height) consisting of adequate quantity of larval food for their eventual emergence into adults. The eclosed females held on yeast-added whole wheat flour or semolina were sacrificed as virgins in preoviposition state within 24 h of their emergence or as egg laying mateds on the 5th day of their adult life to obtain their ovaries (of one side only) made available by dissections performed on a clean microslide containing minute amounts of distilled water under a stereoscopic binocular microscope. Fresh weight of these ovaries removed from each of such 5 females in the different age groups associated with whole wheat flour or semolina were quickly determined separately on a single pan semi-micro electrical balance and the mean fresh weight of this reproductive tissue in respect of each experimental condition was calculated.

For evaluating the reproductive capacity of females reared on these two diets, male and a female beetle, both associated with the same food and freshly emerge from single-paired pupae were coupled to facilitate mating and subsequer oviposition by the females. These tests were performed in glass vials similar to tho described already for accommodation of pupae for their metamorphosis into adult The diets, given in sufficient amounts and replenished only at the end of 20 days the paired beetles inside the vials were identical to those fed by them during the larval lives. Oviposition was monitored daily for a period of 40 days since emergen of these insects into adults. The decision to terminate the experiments at the end an arbitrarily selected 40-day duration, evidently for practical convenience, wa mainly based on the already known facts about the extended longevity of thes beetles (Feakin 1976; King and Dawson 1977). The hatchability of the eggs laid b the females was also noted.

Investigations into the second aspect entailed utilization of single pairs of newbor male and female insects raised in the laboratory, as in previously described tests, o whole wheat flour supplemented with yeast and provision of one of the followin food items fortified with a specified amount of yeast (w/w) during their imaginal live (i) normal whole wheat flour with or without 5% yeast or only yeast, (ii) whol wheat flour extracted in distilled water, chloroform, diethyl ether or 100% ethanc (extraction procedure using solvent extraction assembly same as already describe earlier with the difference that for each extraction 10 g of flour and 200 ml of solvent were used) and then supplemented with 5% yeast, (iii) certai carbohydrates [starch, sucrose, glucose (BDH, England), fructose (E. Merc Germany)] or a protein [vitamin-free casein (ICN Pharmaceuticals Inc., USA)] or lipid [cholesterol (Sisco Research Laboratories Pvt. Ltd., India)], all enriched wit 5% yeast, (iv) a mixture of some vitamins of the B group [Glaxo Laboratorie (India) Ltd.] consisting of 80 μg of thiamin, 30 μg of riboflavin, 230 μg of niacin, 2 μ of calcium pantothenate and 0·63 μg or 63 μg of folic acid per gram of yeas and (v) certain oilseeds, viz cotton seed (*Gossypium arboreum*), ground nut (*Arach hypogea*), linseed (*Linum usittatisisnum*), sesamum (*Sesamum indicum*), mustar (*Brassica campestris*) or toria (*Brassica napus*) or some spices such as cardamor (*Ammonum subulatum*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygiu aromaticum*), coriander (*Coriandrum sativum*), black pepper (*Peper nigrum*) or cumir seed (*Cuminum cyminum*) each material mixed with 5% yeast.

Every diet, before offering it to these tenebrionids for feeding, was powdered an sieved properly so as to maintain uniformity in its particle size. With the exception c fructose which, on account of its hygroscopic nature, was replenished daily, all othe foods were renewed only once (on the 10th day) during the experiment. The tenure c these oviposition trials, whose general layout was similar to that described in th previous set of tests, was, however, limited to 20 days following emergence of th adults. As before, egg viability was also recorded.

3. Observations

3.1 *E. vittella*

A break-up of the oviposition data indicating summarized information about certai specific features of this reproductive activity in this moth in relation to larval rearin

Table 1. Data relating to length of oviposition period (LOP), peak oviposition day (POD), mean oviposition period (MOP) and mean egg number (MEN)/mated female in *E. vittella* in pairs reared on whole fruit of okra or on its components.

Rearing medium — okra fruit	Number of individuals tested	LOP*		POD**	MOP	MEN
		Min	Max			
Seeds (separated from fruit)	12	12(2)	16(3)	1(83)	14·4	882
Whole fruit	10	3(1)	14(3)	1(77)	10·1	625
Mesocarp	12	6(1)	12(2)	1(56)	10·1	375
Epicarp	15	9(1)	15(3)	6(18)	12·8	151

*Figures in parentheses relate to number of females showing the corresponding minimum (min) and maximum (max) lengths of oviposition period for each rearing medium.

**Figures in parentheses relate to the highest mean number of eggs (adjusted to the nearest integer) laid on the corresponding oviposition day by females for each rearing medium.

on whole fruit of okra or on its components is given in table 1. A greater proportion of females in pairs reared on seeds or on epicarp have a longer oviposition period than counterparts associated with couples grown on other two diets. The mean daily oviposition reached its peak value on the first day of egg laying itself in all the females except those in pairs raised on epicarp diet where it occurred only on the 6th day of oviposition. The overall mean fecundity was highest in females belonging to seed-reared couples and this was nearly 6 times more than the lowest mean fecundity score recorded for individuals reared on epicarp diet.

From the results presented above, one can assume that differences observed in the total length of oviposition period of moths in relation to their rearing media can themselves be responsible as an indirect effect of the dietary variations in the rearing media to produce differences in the total number of eggs laid by the females reared variously. Hence a correct evaluation of the direct effect of the dietary differences in the rearing media on the reproductive potential of these moths will be possible only on the basis of comparison of the total number of eggs laid by the females during the same length of oviposition period. Accordingly, this evaluation was based on the total eggs laid by the females during a period of 10 days when one-half to all of the total number of moths that emerged from each rearing medium completed laying more than 65% of the aggregate number of eggs deposited by them during their oviposition period. The observations (table 2) again clearly indicated the competency of females in seed-reared pairs to lay maximum number of eggs during this stipulated oviposition period. Interestingly, this was significantly different even from the total egg output recorded for individuals raised on whole fruit which also contained its seeds.

Having realized that nutrition obtained during larval life from developing seeds is unquestionably superior to that provided by other parts of okra fruit for *E. vittella* to achieve highest reproductive capability, it was of interest to ascertain the time-bound compulsory requirement for the various seed-resident nutritive constituents for the caterpillar to complete its development and to subsequently function as a sexually mature and efficiently productive adult. Table 3 summarizes the data relating to this aspect of the investigation. Mated females in pairs whose nutrition during the first 3 days of their larval lives was provided by developing seeds and then by ripened seeds of okra were only fit enough to lay significantly greater number of eggs than their

Table 2. Number of eggs laid during the first 10 days of oviposition by mated females of *E. vittella* reared on whole fruit of okra or on its components*.

Rearing medium — okra fruit/components	Number of individuals tested	Mean number of eggs laid
Seeds (separated from fruit)	12	675·1
Whole fruit	5	545·0
Mesocarp	9	355·9
Epicarp	14	119·4
Mean square:		
(i) Treatment		710367·3
(ii) Error		4507·2
F(1%)		157·6

*Data subjected to analysis of variance for samples of unequal size (Snedecor 1961).

Table 3. Egg deposition during the first 4 days of oviposition and hatchability of eggs laid by mated females of *E. vittella* in pairs reared on various okra seed-related dietary regimens (data pooled from 5 females per test)*.

Dietary regimen	Mean number of eggs laid	Mean number of viable eggs deposited
Pairs reared during the first 72 h of their larval lives on normal and tender seeds and later on normal and ripened seeds	267·00 a	227·20 a
Pairs reared during the first 24 h of their larval lives on normal and tender seeds and later on normal and ripened seeds	174·40 b	153·40 ab
Pairs reared during the first 72 h of their larval lives on normal and tender seeds and later on petroleum ether-extracted seeds	170·40 b	102·80 b
Pairs reared all through on normal and ripened seeds	160·00 b	139·40 b
Mean	192·95	155·70
LSD (1%)	105·19	114·74
(5%)	76·34	83·27

*Means in the same vertical column followed by the same letter do not differ significantly at the 1% or 5% level by the least significant difference test (LSD) (Paterson 1939).

counterparts reared on other dietary combinations ($P<0·01$ or $0·05$). Further, hatchability of eggs deposited by these highly fecund females was markedly higher ($P<0·01$ or $0·05$) than that recorded for eggs released by mateds in couples developed from larvae and pupae maintained on remaining dietary regimens tested here excepting the one where the caterpillars during the first 24 h of their lives were deriving nourishment from developing seeds and later from ripened seeds of okra ($P>0·05$).

An examination into how far larval dietary regimen consisting of (i) tender seeds of okra fruit for one sex and epicarp of this fruit for the other sex or (ii) ovary of hoeflower would affect egg deposition and egg viability in this noctuid species yielded additional interesting results. The findings (table 4) compared with such data

Table 4. Estimates of egg output and egg viability in *E. vittella* in tests where males and females of copulating pairs were held during rearing, on varied or on similar dietary regimens (data pooled from 5 females per test)*.

Dietary regimen	Mean number of total eggs laid	Mean number of viable eggs laid
Okra fruit—developing seeds for both sexes	243·80 a	176·40 a
Okra fruit—epicarp for both sexes	158·00 b	70·60 b
Okra fruit—epicarp for males and developing seeds for females	154·80 b	107·60 b
Okra fruit—developing seeds for males and epicarp for females	145·20 b	75·60 b
Ovary of shoeflower for both sexes	103·00 b	80·60 b
Mean	160·96	102·16
LSD (1%)	86·00	80·49
(5%)	63·06	59·01

*Same notations as in table 3.

Table 5. Number of eggs laid by mated females of *E. vittella* in pairs fed on different carbohydrate diets or distilled water during their adult lives (data pooled from 5 females per test)*.

Adult food	Mean number of eggs laid
Raffinose	604·6 a
Galactose	509·8 ab
Sucrose	445·4 b
Fructose	420·4 bc
Glucose	317·4 c
Distilled water	164·8 d
Mean	410·4
LSD (1%)	155·1
(5%)	114·5

*Same notations as in table 3.

rocured from moths where both sexes were raised on developing seeds or on picarp clearly revealed statistically significant increments in mean egg output and gg viability in females following mating between the sexes reared exclusively on kra seed diet. Nourishment obtained by pairs from other food combinations tested ere gave rise to mateds having pronouncedly low and statistically similar eproductive capacity.

The influence of adult diet (one of various selected sugars or plain distilled water) n the reproductive potential of this insect was also assessed. Notwithstanding bsence of any major difference in longevity or in length of oviposition period etween moths ingesting a carbohydrate diet and those feeding on water, a ignificantly greater number of eggs were laid by the females fed on any one of the est sugars than on water (table 5). Amongst the various carbohydrates, raffinose at 5% strength helped the individuals to deposit maximum number of eggs and viposition by the moths was equally at a significantly higher level when they ngested this trisaccharide even when maintained at as low a concentration as 1% $P < 0{\cdot}01$) (table 6).

Table 6. Number of eggs laid by mated females of *E. vittella* in pairs fed on different concentrations of raffinose solutions during their adult lives (data pooled from 5 females per test)*.

Concentration	Mean number of eggs laid
15·00	604·6 a
5·00	569·6 a
1·00	534·6 a
0·50	316·2 b
0·25	293·2 b
0·10	254·8 b
Mean	428·8
LSD (1%)	147·7

*Any two means followed by the same letter do not differ significantly at the 1% level by the least significant difference (LSD) test.

Table 7. Number of eggs laid by mated females of *E. vittella* in pairs raised on epicarp or developing seeds during pre-imaginal stages and adults given raffinose or glucose solution respectively (data pooled from 5 females per test).

Experimental condition	Mean number of eggs laid (± SE)
*Epicarp-reared and raffinose given in adult stage	427·2 ± 18·66 [a]
*Seed-reared and glucose given in adult stage	322·0 ± 20·50

*Okra fruit component.
[a]Significantly different at 1% level from the value just below in the column (*t* test) (Paterson 1939).
SE, Standard error.

In the light of informations presented above, egg yield values in this moth was compared when raffinose was provided as adult food for pairs whose immature stages were reared on epicarp diet with couples fed on glucose during their imaginal lives and whose immature stages were raised on developing okra seeds. Number of eggs deposited by moths associated with the former dietary regimens was decidedly greater ($P < 0{\cdot}01$) (table 7) than that laid by those related with the latter set of foods.

3.2 *T. castaneum*

Mean fresh weight of ovaries of females reared and fed on whole wheat flour, whether unmated and remaining in preoviposition state (< 24 h old beetles) or mated and ovipositing (5-day old beetles), was comparatively higher than that determined for counterpart individuals that ingested semolina following their development on the same diet (table 8). Also, egg output in these beetles was more if they were held, right from their birth, on whole wheat flour instead of on semolina (figure 1).

Females whose adult food, like those of males which mated with them, consisted of

Table 8. Weight (μg) of ovaries (left side only) of unmated or mated females of *T. castaneum* reared and maintained on whole wheat flour or semolina supplemented with yeast (data pooled from 5 females per test).

	Mean fresh weight of ovaries (± SE)	
Diet + 5% yeast	Unmated[a]	Mated[b]
Whole wheat flour	180 ± 37·51	600 ± 70·90
Semolina	140 ± 24·56	540 ± 51·12

[a] < 24 h old females in preoviposition state.
[b] 5 day old ovipositing females which mated with males belonging to the same dietary regimen.
SE. Standard error.

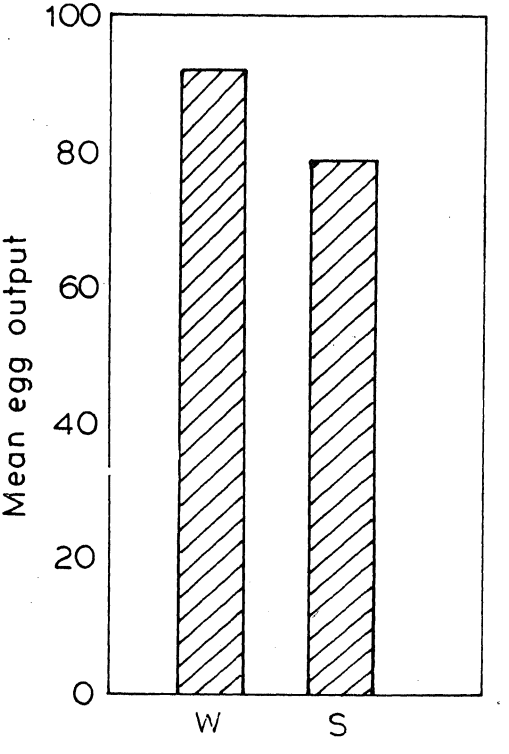

Figure 1. Histogrammic representation of mean egg output by a mated female of *T. castaneum* in a pair reared on whole wheat flour (W) or semolina (S) enriched with yeast and adults maintained during a 40-day experimental period on the same diet as their larvae (data pooled from 5 females per test).

whole wheat flour extracted in 100% ethanol instead of in water, ethyl ether or in chloroform and then reinforced with yeast showed, subsequent to mating, maximal egg deposition possessing high statistical significance (table 9). Curiously enough, this egg yield value was significantly higher ($P < 0{\cdot}01$) than that obtained from mateds allowed to eat normal whole wheat flour fortified with yeast.

Table 9. Number of eggs laid during a 20-day period, commencing from the day following emergence, by mated females of *T. castaneum* in pairs fed on normal or differently extracted whole wheat flour during their adult lives (data pooled from 5 females per test)*.

Diet (enriched with 5% yeast)	Mean number of total eggs laid
100% ethanol-extracted whole wheat flour	131·20 a
Normal whole wheat flour	57·20 b
Ether-extracted whole wheat flour	13·80 c
Water-extracted whole wheat flour	10·60 c
Chloroform-extracted whole wheat flour	8·80 c
Mean	44·32
LSD (1%)	22·56
(5%)	16·54

*Same notations as in table 3.

Low productivity by females compelled to feed on flour extracted in water, chloroform or ether, despite these diets supplemented with yeast, might possibly be due to this cereal getting depleted, consequent to such treatments, of a variety of useful substances like carbohydrates, proteins, lipids and vitamins, many of which are likely to be of great nutritional value for these beetles in regulating their total egg output as in several insects (Wigglesworth 1960; Johansson 1964; Engelmann 1970; Adiyodi and Adiyodi 1974; Nayar 1977). It was, therefore, considered proper to enquire into this issue also wherein the egg laying capabilities of these tenebrionids fed during their adult lives on one of certain specific organic nutrients enriched with yeast were compared with those of counterpart individuals ingesting yeast-containing normal whole wheat flour and the data presented in table 10.

Although normal whole wheat flour proved to be the best amongst the various foods tested enabling the females to exhibit maximum oviposition ($P<0·01$), egg output by individuals fed on starch was significantly higher ($P<0·01$) than that recorded from females in pairs that ate sucrose, fructose, cholesterol or a mixture of certain vitamins of B complex. A similar state of affairs existed when these beetles ingested vitamin-free casein instead of cholesterol or B complex vitamin mixture, the protein nutrient also helping, though to a relatively less extent, the mateds to release significantly more eggs ($P<0·05$) than the disaccharide or the ketohexose monosaccharide compounds.

The lowest egg yield value obtained from mated females of *T. castaneum* where the food of both sexes consisted of yeast supplemented vitamin B complex mixture (table 10) was suspected to be the outcome of one or the other essential vitamin components occurring in alarmingly low proportions in the mixture. Preliminary study involving casual observations indicated an increased egg laying by the copulated females if the beetles ingested vitamin B complex mixture containing higher amount of folic acid. This led to an examination of this phenomenon in greater detail when the folic acid content in the vitamin food was augmented by 100 times (63 μg/g of yeast diet) and the number of eggs laid by females in couples fed on yeast-mixed B complex mixture having low or high folic acid was compared with that deposited by mateds

Table 10. Number of eggs laid during a 20-day period, commencing from the day following emergence, in mated females of *T. castaneum* in pairs fed on whole wheat flour or on different organic nutrients during their adult lives (data pooled from 5 females per test)*.

Diet (all excepting vitamin B complex mixture**) enriched with yeast	Mean number of total eggs laid
Whole wheat flour	57·20 a
Starch	19·60 b
Vitamin-free casein	19·00 b
Glucose	9·40 bc
Sucrose	4·80 c
Fructose	4·40 c
Cholesterol	2·40 c
Vitamin B complex mixture**	1·80 c
Mean	14·82
LSD (1%)	14·62
(5%)	10·87

*Same notations as in table 3.
**For details concerning preparation and composition of this diet, see § 2.

Table 11. Number of eggs laid during a 20-day period, commencing from the day following emergence, by mated females of *T. castaneum* in pairs fed during their adult lives on whole wheat flour or on vitamin B complex mixture** having low or high folic acid content (data pooled from 5 females per test)*.

Diet	Mean number of total eggs laid
Whole wheat flour + 5% yeast	57·20 a
Vitamin B complex mixture** with high folic acid content (63 μg/g of total diet)	43·40 a
Vitamin B complex mixture** with low folic acid content (0·63 μg/g of total diet)	1·80 b
Mean	34·13
LSD (1%)	21·53
(5%)	15·36

*Same notations as in table 3.
**Same notations as in table 10.

maintained on whole wheat flour plus yeast. A significantly greater egg laying was noticed in females in tests where the beetles fed on yeast — vitamin B complex mixture with increased folic acid content ($P < 0{\cdot}01$) (table 11). Such a shoot-up in the egg number of these females brought them, statistically, at par with individuals whose adult food comprised normal whole wheat flour mixed with yeast ($P > 0{\cdot}05$).

All egg yield data from mated females of *T. castaneum* put to variable nutritional experiences, as outlined above, were obtained when yeast was never omitted in the marginal food of these beetles. Whether such inclusion of yeast in a diet fed by these insects is mandatory to improve the egg output of females and whether individuals

that ingest only yeast can exhibit reproductive efficiency similar to those eating food with or without this ingredient are questions demanding clearer answers though Sokoloff and Ho (1962) were the first to throw some light on this matter. A probe into these issues made in the present investigation indicated a definite rise in the egg output by females if they belong to pairs whose whole wheat flour diet, instead of being offered as such, was supplemented with yeast ($P<0{\cdot}05$) (table 12). However, yeast alone as an adult food was miserably incompetent to induce a greater oviposition in these mateds in comparison to even those that were maintained on plain whole wheat flour ($P<0{\cdot}01$).

Results concerning the effect of different oilseeds or whole wheat flour supplemented with yeast fed by adults of this pest on the insect's oviposition are displayed in table 13. A significantly higher number of eggs were deposited by females associated with pairs that ingested groundnut or cotton seed supplemented with yeast instead of other oilseeds. No statistical difference was observed in the oviposition between beetles maintained on groundnut and whole wheat flour both fortified with yeast, the latter diet, nonetheless, stimulating a markedly greater egg

Table 12. Number of eggs laid during a 20-day period, commencing from the day following emergence, by mated females of *T. castaneum* in pairs fed during their adult lives on whole wheat flour with or without yeast or on yeast alone (data pooled from 5 females per test)*.

Diet	Mean number of total eggs laid
Whole wheat flour + 5% yeast	57·20 a
Whole wheat flour	36·80 b
Yeast	1·60 c
Mean	31·86
LSD (1%)	20·47
(5%)	14·67

*Same notations as in table 3.

Table 13. Number of eggs laid during a 20-day experimental period, commencing from the day following emergence, in mated females of *T. castaneum* in pairs fed on different oilseeds or whole wheat flour during their adult lives (data pooled from 5 females per test)*.

Diet (enriched with 5% yeast)	Mean number of total eggs laid
Whole wheat flour	57·20 a
Ground nut	42·20 ab
Cotton seed	35·00 b
Sesamum	17·40 c
Linseed	15·80 c
Mustard	4·20 c
Toria	4·20 c
Mean	25·14
LSD (1%)	29·84
(5%)	22·12

*Same notations as in table 3.

laying in comparison to cotton seed ($P < 0{\cdot}05$) or remaining oilseeds ($P < 0{\cdot}01$). But sesamum, in which postembryonic development of these beetles was unsuccessful (Pajni and Virk 1978), when eaten by these insects during their adult lives, facilitated the mated females to lay eggs which, interestingly, was about 50% of that recorded from females in pairs whose food was cotton seed plus yeast.

Amongst the various yeast-supplemented spices tested, only coriander enabled these beetles to be somewhat productive—the mean number of eggs laid per female being 10·8. All the remaining diets in this category proved incompetent even to sustain longevity of these tenebrionids till the close of the 20-day experimental period.

Egg viability in all the trials arranged here was 100%.

4. Discussion

4.1 *E. vittella*

A conspicuous contrast in the reproductive efficiency of this pest was noticed between females associated with pairs reared on tender okra seeds (0–8 days old) and those in couples grown on epicarp of this fruit or on ovary of shoeflower. A profound influence of larval nutrition on egg production in the adult (Wigglesworth 1960) is implicit in these findings. The considerably high fecundity exhibited by the moths developed on seeds is evidently due to the effect of a superior nutritional quality of the diet, at least in regard to free amino acids and water-soluble proteins (Mani *et al* 1986) which, in addition to accelerating the overall development (Mehta and Saxena 1973; Vishwapremi and Krishna 1974a), boosts the reproductive potential as well. These nutrient reserves are plausibly accumulated more readily in relatively larger quantities during larval life by the adult females reared solely on seeds and subsequently utilized by them resulting in their greatly enhanced egg output. The fact that the number of eggs laid by females developed on whole fruit, in which such seeds were also present, was still significantly less than that deposited by individuals reared only on seeds separated from the fruit lends additional support to this postulation. That such nutrition becomes an obligatory requirement for the caterpillars, at least for the first 3 days of their lives, to enable the resultant females to become maximally productive can be inferred by comparison of egg yield and egg hatchability values obtained from these individuals, whose subsequent larval and pupal phases were allowed to continue on ripened okra seeds (9–11 days old), with those recorded from females associated with pairs held on other single or mixed dietary regimens for different prescribed periods of their postembryonic development. Further, the mandatory need for both sexes of a copulating pair to avail nutrients from okra seeds during their larval development to facilitate this noctuid species to achieve highest reproductive potential (as manifested through significant increments in mean egg output and mean egg viability in the female belonging to this test couple) clearly indicates that not only in females but also in males is there an apparent impact of nutrition on the reproductive activity, once considered to be of negligible magnitude in insects (Wigglesworth 1960).

So far as the role of adult nutrition in the regulation of reproduction in *E. vittella* was concerned, the results showed a marked elevation in egg deposition when the

adult moths were fed on a sugar solution instead of plain water, although they were all reared under identical conditions and did not have any pronounced difference in longevity or in length of oviposition period. This unquestionably suggests that carbohydrate nutrient is very essential during imaginal period in promoting the egg laying capacity of mated females as in the butterfly; *Colias philodice eurytheme* (Stern and Smith 1960) plausibly through better utilization of dietary protein reserves in yolk formation during egg production (Engelmann 1970). The greatest contrast in the aggregate number of eggs deposited between raffinose- and glucose-fed moths further implies that, like in the syrphid *Sphaerophoria scutellaris* (Lal and Haque 1955), different sugars variably regulate the reproductive potential of the spotted bollworm. The higher egg output by females ingesting fructose or galactose instead of glucose, notwithstanding that all the 3 sugars are monosaccharides, corroborates this interpretation. The maximum egg output by moths fed on raffinose can then reasonably be assumed to be due to the combined effect of fructose, galactose and glucose (all of which will be obtained from the ingested raffinose subsequent to its complete hydrolysis) on the reproduction of this pest. The fact that eggs deposited by moths that imbibed raffinose in adult stage but reared on epicarp diet was significantly greater than those laid by seed-reared and glucose-ingested individuals is a pointer to this possibility and highlights the importance of this trisaccharide as an efficiently compensating nutritional factor in the diet of this insect.

Although the need for a carbohydrate nutrient during adult life to realize full fecundity in females has now been experimentally established in *E. vittella*, it is still not known whether variations in the effect of different sugars ingested by the adults on the female's oviposition are due to superior nutritional value of some of these saccharides or on account of disparities in food quantities consumed. Also the question whether the moths in nature obtain through feeding all the necessary sugars, quality- and/or quantitywise, from the foliage or flowers of one or more of the plants naturally infested by them or they are impelled by instinct to visit certain other plants falling outside their normal host range to fulfil their carbohydrate requirements for reproduction is still open.

4.2 *T. castaneum*

The possession of more heavily weighing ovaries and occurrence of higher egg output by mated females, held right from their birth, on whole wheat flour supplemented with yeast instead of on similarly enriched semolina clearly indicate that the former cereal foodstuff is the most favourable diet for *T. castaneum* so far as its reproductive potential is concerned—an observation which supports the earlier findings of Good (1933) and Hamalainen and Loschiavo (1977) who, in their publications have, however, compared the fecundity of this insect on natural and enriched whole wheat flour with cereal products other than semolina.

The observation that whole wheat flour reinforced with yeast, as a food is relatively nutritionally superior to semolina to effectuate the red flour beetle mated females to lay more eggs evidently led to an examination of the ability of these females to oviposit if this flour, after being extracted in one or the other of different solvents, was provided as adult food to reproductive pairs with addition of yeast. It was found that females in pairs fed on 100% ethanol-extracted whole wheat flour

were capable of depositing the highest number of eggs significantly surpassing the egg output figures obtained not only from counterpart mateds ingesting this cereal food subsequent to extraction in water, ether or in chloroform but also, interestingly, even from those eating yeast-added normal whole wheat flour. While no forthright statement can be made presently to explain this unexpected shootup in the number of eggs laid by mateds fed during their adult lives on ethanol-extracted whole wheat flour, it is, however, presumed that such a chemical treatment of this cereal aided in the removal of certain not yet characterized ingredients that possibly function as natural checks for the females in their reproduction, specifically in relation to oviposition. This postulation may also serve as a basis for explaining, in part, the occurrence of poor egg laying in adult females associated with couples ingesting water-, ether- or chloroform-extracted whole wheat flour wherein such detrimental ethanol-soluble constituents were fully present with the simultaneous depletion of several nutritionally important chemical compounds, specially starch and casein, both of which were found to be very important in the reproduction of this insect (table 10). If this assumption is correct, it would not be out of place here to suggest that future investigations into the reproductive biology of *T. castaneum* may be focussed towards chemical characterization and identification of these ethanol-soluble constituents and their judicious application in pest management programmes aimed at limiting the population build-up of this beetle on stored commodities. The poor fecundity (in terms of egg deposition scores) exhibited by the mated females of this species when both sexes, as adults, were fed on water-, chloroform- or ether-extracted whole wheat flour enriched with yeast, notwithstanding ingestion of yeast-added whole wheat flour during their larval life, indicates absence of any benefit provided to the imagines in their reproduction via larval nutrition and thus supports the inference drawn along similar lines by Reynolds (1944) in his studies on *T. destructor*.

Oviposition was significantly curtailed if the adult food of the mated females consisting only of yeast-supplemented B complex vitamins had folic acid content as low as 0·63 μg instead of 63 μg/g of total diet. It appears that folic acid in appreciable concentration is also a necessary constituent in the adult diet of *T. castaneum* for aiding the beetle's reproduction like other components of B vitamins such as riboflavin, thiamin and niacin, all 3 of which have been reported by Hamalainen and Loschiavo (1977) to increase the fecundity of this species.

Addition of yeast to whole wheat flour given as adult diet caused a marked improvement in the egg output by the mated females. From this it is inferred that yeast, in which existence of some growth factors necessary for normal development of *T. confusum* has already been discovered (Charbonneau and Lemonde 1960a, b), presumably contains also certain as yet unidentified components responsible, additively, for the promotion of oviposition in *T. castaneum*.

The reproductive potential of these beetles, subsequent to feeding them on different selected oilseeds or spices in their adult lives, did not always coincide with the comparative dietary efficiency of these products for the larval growth of this insect (Pajni and Virk 1978). This implies the occurrence of variability in the nutritional competency of these materials insofar as larval development and adult reproduction in this pest are concerned.

A 100% viability of eggs in all test conditions clearly indicates absence of any effect of nutritional difference on egg hatchability in this tenebrionid species.

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Relation between feeding and egg production in some insects

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Abstract. Food consumption and fecundity of insects vary with life style and feeding pattern. In general, species which feed during the larval and adult stages and maintain a smaller biomass, allocate a higher percentage of the ingested energy to egg production (e.g. *Oryzaephilus surinamensis*; 34·4%). Species which grow larger but feed at lower rates and pass through extended adult life span display very low egg production efficiency (e.g. *Poecilocerus pictus*; 0·7%). A few others feed at faster rates during the larval period, shorten the adult phase and allocate fairly a high percentage of the ingested energy to egg production (e.g. *Bombyx mori*; 5·8%). Food quality regulates food consumption and thereby significantly influences egg production in several polyphagous insects. Prey density influences food consumption and fecundity of predators. Regression of fecundity on blood meal ingested for two hemipterans and two dipterans revealed that the dipterans are autogenous and are relatively independent on adult blood meal for oviposition in comparison with the hemipterans.

Keywords. Feeding pattern; fecundity; egg production efficiency.

1. Introduction

Insects follow a variety of life styles and feeding patterns, which play a major role in determining the strategy of growth and reproduction (Enders 1976; Lawton and McNeill 1979). Southwood *et al* (1974) considered that the generation time of an individual determines its reproductive strategy. Life span of insects varies from a few days in several lepidopterans and parasitic hymenopterans to over 17 years in cicada (Richards and Davies 1977). Some are short lived and are smaller in size (e.g. parasites, aphids, scale insects etc), while a few others are long lived and large sized (e.g. scarabaeid beetles, grasshoppers and locusts etc). A majority of the insects feed throughout life (e.g. most of the ametabolous and hemimetabolous insects and a few holometabolous insects), while several lepidopterans, ephemeropterans and chironomids feed only during the larval period. While a few sanguivores are unautogenous and require an adult blood meal to commence oviposition (e.g. *Aedes aegypti*), several others are autogenous and are independent of adult blood meal for oviposition. Egg production is ultimately an aspect of conversion of ingested food. Therefore, biotic and abiotic factors, which are known to alter the rate and efficiency of food utilization in females also influences her fecundity (Muthukrishnan and Pandian 1987). Although a good number of publications report fecundity of insects, very few alone relate to feeding with energy allocation to egg production. This paper aims to critically evaluate the impact of feeding on egg production in some insects.

2. Materials and methods

Following the gravimetric procedure described by Waldbauer (1968), food consumption, growth and egg production in *Mantis religiosa*, *Coccinella*

Table 1. Food consumption and energy allocation to egg production in few insects.

Insect	Food consumption (J/insect)			Growth (J/insect)	Egg production (J/insect)	Egg production efficiency (%)	Remarks	Reference
	Larva	Adult	Total					
Collembola								
O. cincata	—	—	38·3	3·334	1·87	4·9	Moults and feeds throughout life	Testerink (1982)
T. minor	—	—	38·8	4·467	2·80	7·2	Moults and feeds throughout life	Testerink (1982)
Orthoptera								
O. velox	10208	15967	26175	2180	1077	4·1	Feeds throughout life: grows to about 300 mg in 240 days	Delvi and Pandian (1971)
P. pictus	717587	1211704	1929291	48704	13053	0·7	Feeds throughout life: grows to about 6.9 g in 267 days	Delvi (1972)
Dicyoptera								
M. religiosa	39678	55327	95005	14630	5413	5·7	Feeds throughout life: grows to about 2·2 g in 131 days	Muthukrishnan (1980)
Homoptera								
A. fabae			62	32·3	26	41·9	Feeds throughout life: grows to about 1·0 mg in 39 days	Llewllyn and Qureshi (1978)
Lepidoptera								
B. mori	59469	0	59469	18245	3467	5·8	Feeds only during larval period (25 days), spins an expensive pupal case	Hiratsuka (1920)
C. leucosticta	189083	0	189083	33773	7160	3·8	Feeds only during larval period (107 days) and attains a maximum body weight of 5.1 g	Mackey (1978)
Coleoptera								
C. transversalis	226	964	1190		345	29·0	Feeds throughout life: 4 days larval period: 28 days adult period	Muthukrishnan and Senthamizhselvan (unpublished results)

M. sexmaculatus	292	1424	1716	126·4	389	22·7	Feeds throughout life: 4 days larval period: 32 days adult period	" "
C. cautena		5547	5547	385	296	5·3	Larval consumption not estimated: adult life span 25 days	Senthamizhselvan (1987)
R. verreauxi	124880	0	124880	11140	5414	4·3	Feeds only during larval period (924 days): attains maximum growth of 3·3 g	Cairns (1982)
O. surinamensis	38	273	311	120	107	34·4	Feeds during larval (13 days) and adult period (60 days): attains maximum growth of 25 mg	White and Sinha (1981)
S. granarius	331	1189	1520	87	39·4	2·6	Feeds during larval (19 days) and adult periods (30 days): attains maximum growth of 7 mg	Campbell *et al* (1976)
C. ferrugineus	25·5	310	336	3·2	21	6·0	Feeds during larval (20 days) and adult periods (123 days): attains maximum growth of about 0·7 mg	Campbell and Sinha (1978)
Callasobruchus maculatus	386	0	386	87	21·0	5·4	Feeds only during larval period (16 days); oviposits for 8 days: attains maximum growth of 4 mg	Chandrakantha (1985)

transversalis, *Monochilus sexmaculatus* and *Cicindela cautena* were estimated in terms of energy. However, rest of the information presented in this paper were collected from pertinent publications. From a survey of over 100 publications, about 30 were selected; the others which do not provide quantitative (mass or energy) data for both food consumption and fecundity were not considered. Information presented here pertain to a wide range of insects following different life styles and feeding patterns. The data collected have been discussed in the light of theoretical information provided by Calow (1977).

3. Effect of life style and feeding pattern

Table 1 presents data on food consumption and energy allocation to egg production in a few insects, which follow different life styles and feeding patterns. Egg production efficiency is expressed as percentage of ingested energy allocated to egg production; the efficiency ranges from 0·7% in the grasshopper *Poecilocerus pictus* to 34 and 42% in the granivorous *Oryzaephilus surinamensis* and the juice feeding *Aphis fabae*, respectively. The wide range in the efficiency may partly be attributed to the life style (living for longer or shorter durations, and growing smaller or larger) and feeding pattern (feeding throughout or part of the life span). The primitive collembolans *Orchesella cincata* and *Tomocerus minor* moult throughout life comprising intermoult periods of active feeding and reproduction, and short non-feeding moult periods. About 5 and 7% of the ingested energy (*ca* 38 J) is allocated by them respectively, to egg production. *T. minor* is metabolically less active than *O. cincata* and hence affords to allocate a higher percentage of ingested energy to egg production (Testerink 1982). Of the two species of grasshoppers considered, *Oxya velox* displays egg production efficiency of 4·1% as against 0·7% by *P. pictus*. Although their life span is around 250 days, energy costs of growing larger (6·9 g in *P. pictus* vs 0·3 g in *O. velox*) and maintenance of a larger adult biomass for a longer duration (> 105 days in *P. pictus* vs < 75 days in *O. velox*) have restricted the egg production efficiency of *P. pictus* to the lowest level (0·7%). Metabolic cost of maintenance of larger biomass of adult for a longer duration depletes the energy available for egg production (Calow 1977). Although the scarabaeid beetle *Rhopaea verreauxi* lives longer (924 days) than *P. pictus* and attains a biomass of 3·3 g, it allocates 4·8% of the ingested energy to egg production. It does not feed as adult but converts most part of the energy accumulated during the larval period into egg. On the other hand, *P. pictus* allocates a part of the ingested energy during nymphal period to somatic growth and depends mostly on the energy ingested during the adult period for maintenance and egg production. The strategy of the moth *Cyclophragma leucosticta* is similar to that of *R. verreauxi*. Despite growing as large as 5·1 g and passing through a larval period of 107 days, *C. leucosticta* allocates 3·8% of the ingested energy to egg production. *Bombyx mori* spins an expensive pupal case and yet manages to spare 5·8% of the ingested energy for egg production. Granivorous beetles like *Sitophilus granarius*, *O. surinamensis* and *Cryptolestes ferrugineus* adopt a different strategy. They resort to shorten the larval period susceptible to parasitic infection and prolong the adult period to acquire sufficient energy and nutrients and maximise egg production. Shortening the larval period to 4 days and decreasing the adult biomass to 13·9 mg have helped the aphidophagous

ladybird beetles *C. transversalis* and *M. sexmaculatus* to allocate as much as 29·0 and 22·7% of the ingested energy to egg production. Ingestion of amino acid-rich phloem sap enables *A. fabae* to enhance its egg production efficiency to 41·9%.

4. Effect of quantity of food

In most hemimetabolous insects, quantity of food ingested during the adult period plays a major role in reproduction and hence restriction of adult feeding is likely to affect egg production efficiency. Table 2 provides data on food consumption and egg production by the milkweed bug *Oncopeltus fasciatus* receiving 100, 50 and 25 mg dry seeds/week/pair (Slansky 1980). A female in 100 mg ration group passed through interoviposition periods of 2·3 days and oviposited 1217 eggs weighing 119 mg in total. Owing to the extension of the interoviposition period to 4·7 and 10·0 days and the consequent increase in the metabolic cost of maintenance, egg production efficiency of females in the 50 and 25 mg ration groups decreased to 21·1% and 7·7%, respectively. Corpora allata of females receiving restricted rations are also likely to have not been sufficiently activated resulting in decreased fecundity (Ralph 1976). Restriction of ration may also directly interfere with egg production and decrease fecundity (Walker 1976). Mathavan (personal communication) has obtained a highly significant correlation between feeding rate and fecundity on the one hand and faeces egested and fecundity on the other for the silkworm *B. mori.* The simple linear regression equations especially the one between faeces egested and fecundity developed may be useful to predict fecundity of *B. mori* (figure 1).

Discussing stability and resilience in predator—prey models, Beddington (1976a, b) showed that prior to commencement of oviposition, a predator should consume sufficient energy to meet its metabolic demand; once this demand is met, fecundity of the predator holds a definite relation to prey ingested by it. For instance, *Coccinella undecempunctata aegyptiaca* consumes about 25 aphids/day before commencing oviposition; thereafter, with increasing prey consumption to 40 and 75 aphids/day, fecundity increases to 60 and 160 eggs/female (table 3). Dixon (1959) has also reported similar relation for the beetle *Adalia decempunctata* (table 3).

5. Effect of food quality

In addition to differences in chemical composition, natural food materials differ in their capacity as phagostimulants. Consequently, quantities of food consumed,

Table 2. Effect of ration levels on food consumption and egg production in *O. fasciatus* fed on air-dried seeds of *Asclepia syriaca* for a period of 13 weeks after eclosion (from Slansky 1980; modified).

	Ration (mg/seed/week/♀♂)		
Parameters	100	50	25
Consumption (mg dry wt/♀♂)	422·4	268·2	189·8
Inter-oviposition period (day)	2·3	4·7	10·0
Fecundity (egg/female)	1217	576	131
Biomass of eggs produced (mg dry wt/♀)	119·0	59·4	14·6
Egg production efficiency (%)	27·9	21·1	7·7

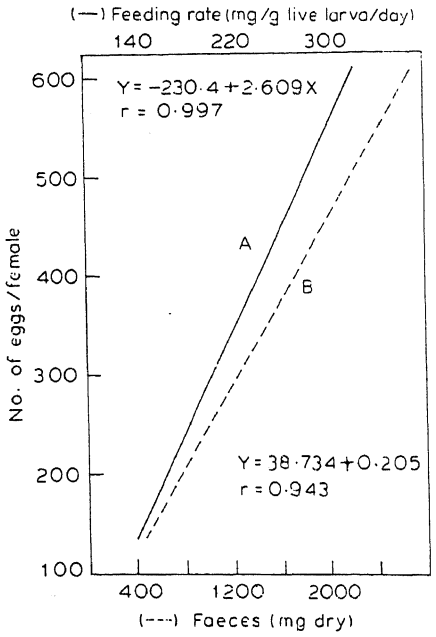

Figure 1. Fecundity of *B. mori* as functions of (A) feeding rate and (B) faeces egested (Mathavan, personal communication).

Table 3. Fecundity in relation to prey density in *C. u. aegyptiaca* and *A. decempunctata.*

C. u. aegyptiaca[a]		*A. decempunctata*[b]	
Prey density (No./♀)	Fecundity (egg/♀)	Prey density (No./♀)	Fecundity (egg/♀)
30	10	3	3
35	30	7	13
40	60	15	18
55	120	23	15
60	130		
75	160		

[a]Data from Beddington *et al* (1976a). [b]Data from Dixon (1959).

assimilated and converted by polyphagous insects vary with quality of food (Soo Hoo and Fraenkel 1966; Muthukrishnan and Rajeeya 1979). Such differences in food utilization account for the variations in the fecundity of a number of polyphagous insects. For instance, fed ad libitum on *Solanum tuberosum* and *Mamestra configurata* consumed 388 mg (dry) of food and produced 279 eggs after emergence (Bailey 1976). Feeding on *Brassica campestris* enhanced its consumption to 498 mg and fecundity to 756 eggs (table 4). Young *et al* (1950), East (1977) and Ottens and Tood (1979) have reported wide variations in the fecundity of whitefringed beetles *Graphognathus* spp. fed on leguminous and graminaceous plants. Fecundity of the beetle varied from 4–26 eggs/female while feeding one or the other of 12 different species of grass to 153–631 or 1450 eggs/female for those feeding leguminous or malvaceae plants

Table 4. Effect of food quality on food consumption and fecundity of *M. configurata* (from Bailey 1976; recalculated).

Host plant	Food consumption (mg dry wt/larva)	Fecundity (egg/♀)
S. tuberosum	388	279
Cheuopidium album	448	411
B. campestris	498	756
Brassica napus	494	838
Artificial diet	—	1367

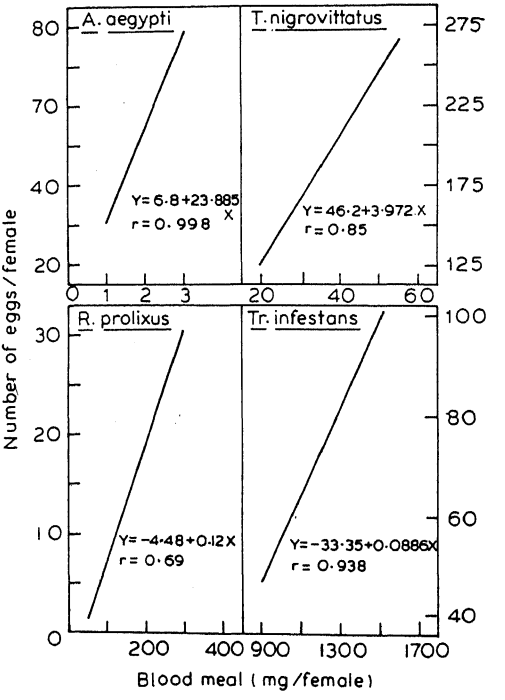

Figure 2. Fecundity of some sanguivorous insects as a function of blood meal consumed. (**A**) *A. aegypti* (Roy 1936); (**B**) *T. nigrovittatus* (Magnarelli and Stoffolano 1980); (**C**) *R. prolixius* (Patterson 1979); (**D**) *T. infestans* (Regis 1979).

(Ottens and Tood 1979). However, food consumption in the beetles feeding on different host plants was not estimated by the authors and hence fecundity could not be related with quantity of food consumed.

6. Autogeny

By feeding the stable fly *Stomaxys calcitrans* on a blood meal mixed with soybean trypsin inhibitor, Spates (1979) demonstrated the importance of protein for egg production in sanguivores. However, arctic mosquitoes such as *Aedes impiger* and *A. nigriceps* as well as *A. detritus* inhabiting the deserts of Sahara and Tunisia, where

chances of finding mammalian host are remote are autogenous and produce eggs without having to consume a blood meal after emergence (Corbet 1964). Larval nutrition is one of the major factors controlling autogeny in insects (Friend *et al* 1965). For instance, the bug *Rhodnius prolixus* fed on full blood meal at each nymphal instar oviposits 51 eggs after ingesting first adult blood meal compared with 18 eggs by that fed on partial meals during nymphal period (Patterson 1979). Fecundity of the bug *Triatoma infestans* ingesting 930–1510 mg of blood meal after eclosion varies from 50–95. On an average about 16·6 mg of blood is required for the production of an egg (Regis 1979). Magnarelli and Stoffolano (1980) fed *Tabanus nigrovittatus* on different quantities of blood (0–60 mg) and observed a linear relation between quantity of meal and fecundity. They have demonstrated that the autogenous and anautogenous fecundity of the fly do not differ significantly. Figure 2 shows the relation between blood meal ingested and fecundity for two dipterous and two hemipterous sanguivores. The negative intercept on y of the regression lines for the hemipterous *R. prolixus* and *T. infestans* clearly indicates that blood consumption after eclosion is obligatory for the initiation of oviposition; on the other hand, the positive intercept for the dipterous *A. aegypti* and *T. nigrovittatus* indicates their relative independence on adult blood meal for oviposition of at least a few eggs.

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Nutrient modulation of hormone production: Dietary essential fatty acids, tissue prostaglandins and their probable role in insect reproduction

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Abstract. The essential polyunsaturated fatty acids required by insects in their food appear to be needed for prostaglandinogenesis. Prostaglandins themselves are likely to be widely distributed in insects playing perhaps an hitherto unsuspected important role as in reproduction.

Keywords. Polyunsaturated fatty acids; arachidonic acid; prostagladins; reproduction; insects.

Hobson (1935) found that cholesterol was an essential nutrient for blowfly larvae and Fraenkel and Blewett found that the unsaturated fatty acid linoleic acid was required for proper development and reproduction in many insects (Fraenkel and Blewett 1945, 1946). They also found that arachidonic acid played an important role in the metabolism of insects (Fraenkel and Blewett 1947). Linolenic acid was found to be as effective as linoleic acid, but oleate was ineffective in many insects as in *Ephestia kühniella*, but in others as in *Tenebrio*, linoleate was not required. Apparently, linoleate or linolenate was synthesized by the animal or the symbionts harboured by the insect. This work was followed by a spate of information stressing the requirement of various long chain poly unsaturated fatty acids in the diet of various insects for their normal physiological maintenance. From the vast literature which has accumulated, it was clear that insects, as mammals, cannot synthesize linoleate or linolenate from simpler precursors. However, their functional role in normal growth and reproduction remained largely unknown. Chippendale's work on *Trichoplusia ni* (Chippendale *et al* 1964) showed the necessity of using pure samples for nutritional studies as contaminants had nutritional value. They found that linolenate was an essential nutrient, which could not be replaced by linoleate. It is now well-established that sterols are indispensible nutrients for all insects, and also require dietary polyunsaturated fatty acids wherever they have been studied critically (Chippendale 1972; Dadd 1973; Downer 1978).

Sterols are known to function as structural components of insect membranes, but perhaps a more important role they play is as precursors of the moulting hormone ecdysone (Clayton 1964) and this function of dietary sterol requirement in insects is now well established. However, functional significance of the requirement of very small amount of essential poly unsaturated fatty acids is not yet well understood. As the quantity of these substances required by insects is extremely small they do not appear to be linked with normal energy metabolism.

In the cabbage looper *T. ni*, nutritional studies have shown that either linoleate or linolenate promoted larval growth, linolenate was essential for normal wing development (Chippendale *et al* 1964). Since linoleate and linolenate were preferentially incorporated into phospholipids, a structural role for them in membrane phospholipids was postulated (Grau and Terriere 1971). Dietary

requirement of poly unsaturated fatty acids for successful pupal eclosion, wing expansion, larval growth and reproductive capacity has been detected in many groups of insects (Dadd 1973, 1985; House 1974; Downer 1978). It is difficult to completely eliminate trace amounts of these substances from prepared diets and hence some of the earlier claims of nonessential nature of poly unsaturated fatty acids would require confirmation. These substances may also pass on from mother to offspring; the micro-organisms in the insects may also be involved in the synthesis of these compounds. It is increasingly becoming clear that arachidonic acid, the poly unsaturated fatty acid precursor of prostaglandins in vertebrates, is required for normal growth and development and accelerated larval growth in *Trogoderma granarium* (Pant and Pant 1961) and is essential for emergence of viable mosquito adults (Dadd and Kleinjan 1978) and for eclosion and flight activity of many species of mosquitoes (Dadd and Kleinjan 1979; Dadd 1985).

In vertebrates arachidonic acid and the related C20 polyunsaturates, homo-γ-linolenic and eicosapentanoic acids are precursors of prostaglandins and local hormone-like substances. Several long chain poly unsaturates including arachidonic acid, have also been detected in many species of insects recently (Dadd 1981). Prostaglandins are hormone-like ubiquitous substances whose synthesis and functions are well established in vertebrates (von Euler and Eliasson 1967). Recent detection of prostaglandins and prostagladin synthetase in insects (Destephano and Brady 1977) in the cricket *Acheta domesticus* and further work on this insect points to the likelihood of the important role these compounds might be playing in insect reproduction. Prostaglandins have also been detected in the cricket *Teleogryllus commodus* (Loher *et al* 1981), *Bombyx mori* (Setty and Ramaiah 1979) and in *Musca domestica* (Wakayama *et al* 1980). In all these species, the compound has been found to occur primarily in reproductive tissues though it is also found in other tissues (Murtaugh and Denlinger 1982).

Prostaglandin synthetase was found for the first time in insects in the 12100 g pellet of reproductive tract homogenate of the male cricket *A. domesticus* (Destephano *et al* 1976). The supernatant and microsomal fractions were found to be inactive. Subsequent studies on this animal showed that this enzyme was localised in the testes, seminal vesicles and spermatophores (Destephano and Brady 1977). Non-steroidal anti-inflammatory drugs which are known to inhibit enzymes of the prostaglandin synthetase complex (indomethacin, phenyl butazone and acetaminophen), appeared to inhibit prostaglandinogenesis from arachidonic acid in *Culex pipiens* when the larvae were reared in media containing the inhibitors and arachidonic acid (Dadd and Kleinjan 1984). In *Acheta domesticus* the enzyme was absent in bursa copulatrix, spermatheca, spermathecal canal and oviducts from virgin females, but in mated females substantial enzyme activity was detected in these organs. Destephano and Brady (1977) concluded that the female cricket received the enzyme at the time of mating through spermatophore. PGE_2-like material was found in this animal in the testis and to a lesser extent in the remainder of the male reproductive tract, but was not present in the virgin female reproductive tissues. However, on mating, the reproductive tissues acquired PGE_2-like material. Injection of PGE_1, PGE_2 and $PGF_{2\alpha}$ stimulated oviposition by virgin females. They also observed that the PG synthetase inhibitor N-acetyl-*p*-aminophenol suppressed oviposition in mated females. The authors concluded that post-copulatory PG biosynthesis in the female reproductive tract might be partially responsible for

triggering oviposition in this animal. These observations of Destephano and Brady (1977) have been confirmed essentially by Loher *et al* (1981) in *Teleogryllus commodus*. In addition they found 7 polyunsaturated fatty acids in the spermatheca of virgin female which included 2·2% (of the total fatty acids) of arachidonic acid, the precursor of PGE_2, and to a lesser amount precursors of PGE_1 and PGE_2 and very high concentration of linolenic acid (36·9%). In vitro incubation studies showed that the spermatophore and the female's spermatheca contents incubated with labelled arachidonic acid as substrate, produced PGE_2 and $PGF_{2\alpha}$, the former about twice the rate of the latter. They also found that nanogram quantities of PGE_2 injected into the oviduct stimulated mating-induced egg release. Setty and Ramaiah (1979) isolated PGE_1, PGE_2 and $PGF_{1\alpha}$ and $PGF_{2\alpha}$ from reproductive tissues including testes, vasa deferentia, seminal vesicles and ejaculatory ducts of the silk moth *B. mori*. Both crude and purified PGE and PGF_α fractions were found to have biological activity when tested using guinea pig ileum preparation in vitro. As opposed to the above findings especially of Destephano and Brady (1977) and of Loher *et al* (1981) in crickets, though low levels of prostaglandin-like compounds were detected in reproductive tissues of the cabbage looper *T. ni* adult males and females which increased 3 folds (PGE_2) and 2 folds ($PGF_{2\alpha}$) in the female reproductive tissues after mating, injected PGE_1, PGE_2 and $PGF_{2\alpha}$ had no effect on either pheromone production, calling behaviour or oviposition in this animal. The cricket, prostaglandin synthetase inhibitor N-acetyl-*p*-aminophenol in the diet of adult females did not alter calling behaviour of virgin or mated female of this species, though in the larval diet the treatment lengthened larval stadia but had little effect on reproductive activities (Hagan and Brady 1982). This could be bacause a prostaglandin different from that injected might be the active one in this species. It has to be borne in mind that prostaglandins are a large family of compounds differing in their action from species to species and from one tissue to another, sometimes with diametrically opposite effects; their action also depends often upon the physiological state of the organ and tissues. Indeed more extensive and detailed work is needed on insects to clarify the significance of prostaglandins in reproduction and perhaps on other activities of insects.

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Assessment of neuroendocrine mechanisms of feeding and reproduction in phytophagous insects

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Abstract. In acridids during feeding, the central nervous system receives information through sensilla which in turn integrated incoming information giving the appropriate motor output. Hormones and haemolyph factors like osmotic pressure also limit meal size. In the red cotton bug, *Dysdercus cingulatus* hormones from the neurosecretory cells of the brain induced feeding and the protein food ingested in turn stimulated midgut enzyme production while juvenile hormone has little influence either on feeding or enzyme production. In lepidopterans both brain hormone and juvenile hormone seemed to stimulate feeding and the amount of food ingested induced enzyme production. Midgut endocrine cells have also been demonstrated in several lepidopteran species.

Reproduction involving vitellogenesis, spermatogenesis, oviposition, accessory glands activity and mating behaviour is also under the regulatory control of several hormonal principles such as juvenile hormone, ecdysone, oostatic hormone etc. However, a detailed analysis of the literature available on the topic reveals that these regulatory mechanisms are tissue and species dependent.

Keywords. Feeding; reproduction; neuroendocrines; vitellogenesis; digestion.

1. Introduction

The fundamental nutritional endeavour of any organism is to enumerate their food substances to the simplest utilisable chemical form, to distinguish those which are essential rather than merely usable and to determine the relative amount of each substance that are optimally needed. In that perspective, different physiological processes like feeding, digestion and absorption constitute their own specific roles in preparing the insect finally for meeting their needs for energy and progeny production. Various aspects of insect feeding have been discussed in detail in some of the recent reviews (Gelperin 1971; Truman and Riddiford 1974; Barton Browne 1975; Bernays 1985). The regulation of feeding is part of a larger mechanism for metabolic homeostasis. In addition to classical hormones as brain hormone (BH), juvenile hormone (JH) and ecdysone, several of the other endocrine principles are also found to influence many of the physiological processes related to feeding, nutrition and reproduction in insects. Most of the insects continue to feed throughout their life thus supplying the essential materials for body maintenance, growth and reproduction. Reproduction is the culmination of so many of the sequential processes such as sexual differentiation, gametogenesis, mating, oviposition etc which are again under the influence of different hormones. Many of the recent reviews have highlighted these aspects in sufficient detail (Engelmann 1970; de Wilde and de Loof 1973; Raabe 1982; Davey 1984; Koeppe *et al* 1985; Hagedorn 1985).

2. Feeding and digestion

2.1 *Acridids*

Sectioning of the posterior pharyngeal nerves in *Locusta migratoria* in front

of the foregut region resulted in hyperphagia while sectioning other nerves to the foregut did not show this effect (Bernays and Chapman 1973). During feeding, the central nervous system (CNS) of these insects receives information from a large number of sensilla each of which contain numerous physiologically different receptor cells. CNS integrates the incoming information with input from other sensory system to give the appropriate motor output. The pharyngeal nerves supply the anterior part of the foregut, the last part to be filled during a meal. Here there is a network of nerves containing about 10 large cells probably being the perikarya of stretch receptors and the input from these cells indicating complete fullness of the foregut which is the signal for cessation of feeding (Bernays 1985). Haemolymph factors may also limit meal size. If the osmotic pressure in the haemolymph was elevated without volume change about 20 min before feeding, meal size in *Locusta* larvae reduced substantially suggesting a control by osmotic pressure on feeding (Bernays and Chapman 1974). However, an alteration of nutrient concentration and osmotic pressure during the course of a meal through an implanted cannula had no effect on the size of that meal. An extract of corpora cardiaca (CC) also did not have any effect on the meal size. Further, crop stretching switches off feeding and results in the release of one or more hormones from the storage lobes of CC. One such hormone is the diuretic hormone influencing the absorption of water from the food thus increasing the haemolymph volume (Mordue 1969). A hormonal principle also causes the pores on the terminal sensilla of the palps to close so that the sensilla are no longer functional. This effect as measured by the changes in electrical resistance across the tips of the palps persists for at least an hour in locusts which have had a large meal following a period of deprivation. Two hours later, the sensilla are again fully functional and injections of CC storage lobe extract showed that the change is concentration dependent (Bernays and Mordue 1973). The hormonal principle from the CC storage lobes enhanced the movements of the gut as well as in locusts (Cazal 1969).

In *Melanoplus sanguinipes*, a massive release of neurosecretion from brain median neurosecretory cells (MNC) and CC occurs within 20–30 min of feeding (Dogra and Gillott 1971). Release of neurosecretion is initiated by stretch receptors on the foregut so that frontal ganglionectomy or certain other operations on the stomodaeal nervous system prevents it since the operation results in an interference with a large number of sensory, motor and relay pathways. The movement of food back from the buccal cavity is reduced and it also prevents the forward flow of enzymes from the midgut (Anstee and Charnley 1977). Estimations of dry food eaten by *Locusta* show that the amount consumed by allatectomised males does not differ significantly from that consumed by their controls (Strong 1975). Also implantation of additional corpora allata (CA) in the larvae of *L. migratoria* did not alter food consumption (Beenakkers and Van Den Broek 1974). Hence JH seems to have very little regulatory influence on feeding activity in the Orthopterans.

But midgut enzyme activity is clearly related to food intake in almost all the orthopterans studied and Dogra (1974) in the light of available literature concludes that in this group of insects enzyme production is regulated by a neurosecretion released from brain as a result of crop filling or foregut activity. The timing of release of neurosecretion in *Melanoplus* is such that it could regulate protease activity increase. According to Anstee and Charnley (1977) the enzyme production in locusts is regulated by the total volume of food ingested acting *via* the neurosecretory system. Cauterisation of the MNC of brain in the adult *Gomphocerus rufus* brought

down the protease level significantly (Loher 1965). Release of neurosecretion is initiated by stretch receptors on the foregut so that frontal ganglionectomy or certain other operations on the stomodaeal nervous system prevents it (Clark and Langley 1963) and the gut carbohydrase activity is also reduced (Bernays and Chapman 1973). Neither ovariectomy nor allatectomy affected protease activity in grasshoppers (Dogra 1974) and even in the starved insects a residual level of enzyme activity was detected. Khan (1964) observed an increase in enzyme activity in locusts after moulting and hence some enzyme activity is assumed even in the absence of feeding. Neck ligature performed within 24 h of moulting also reduced invertase activity in the insects even though injection of blood from fed donors did not elevate the enzyme activity in starved recipients.

2.2 *Red cotton bug*

We in our laboratory have tried to delineate the endocrine mechanisms on feeding and digestion in the female red cotton bug, *Dysdercus cingulatus*. Extirpation of MNC soon after their emergence retarded feeding activity while implantation of active MNC from other donors restored this effect to a substantial level (Muraleedharan and Prabhu 1979a). Hence the neurosecretory principles from the brain seem to stimulate food consumption in this bug. But JH was found to have no significant influence on food consumption in this insect (Muraleedharan and Prabhu 1981). Also ovariectomy in these insects resulted in hyperphagia and hyperactivity of median neurosecretory cells in the brain. Also injection of methanol extract of active ovaries retarded rate of feeding in ovariectomised *Dysdercus* which tempted us to conclude that in this insect some ovarian factor (probably ecdysone) inhibited the activity of MNC resulting in the reduced feeding rate (Muraleedharan 1984). Midgut protease and invertase activities were also very low in MNC-ablated cotton bugs while implantation of MNC induced enzyme production. Since casein was found to stimulate protease activity (Muraleedharan and Prabhu 1978) and sucrose fed animals showed only residual levels of protease and invertase activities (Muraleedharan and Prabhu 1979b) it became also apparent that in *D. cingulatus* ingested food especially proteins stimulated midgut enzyme production through a secretogogue mechanism (figure 1).

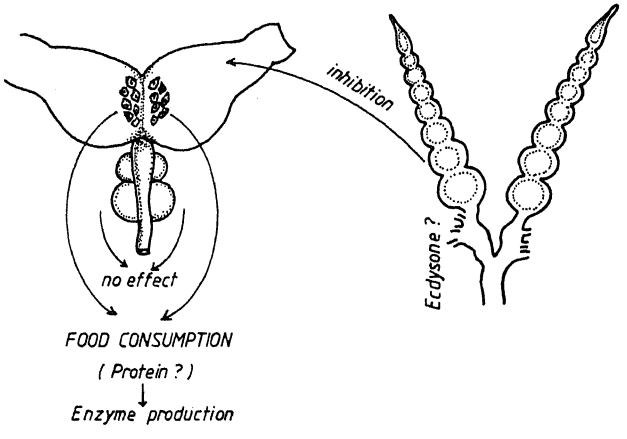

Figure 1. Probable regulatory mechanism of feeding and digestion in *D. cingulatus*.

2.3 *Lepidopterans*

Our own studies in the late instar caterpillars of the teak defoliator, *Hyblaea puera* demonstrated that implantation of fresh and active brain to raise the insect's own neurosecretion stimulated feeding. Additional supply of JH (ZR 515-JHa) through food also induced feeding (Muraleedharan and Prabhu 1981). Higher levels of midgut amylase activity also were noticed in both the above experimental groups of caterpillars. Hence it seems that in this insect both MNC-hormone and JH induced feeding and the food ingested in turn must have stimulated amylase production *via* a secretogogue mechanism. However, these findings do not confirm whether the MNC- hormone stimulated feeding directly or through its trophic action on corpora allata since MNC is known to secrete an allatotropic hormone. In the larvae of the common cutworm *Spodoptera litura* also JH seem to stimulate feeding since Tojo *et al* (1985) demonstrated that application of both JHI and JHII increased feeding activity while allatectomy decreased the duration of final feeding period in final instar larvae. In *Bombyx mori* also Calvez (1981) demonstrated the involvement of JH in both obligatory and facultative feeding period. Caterpillars of *Achoea janata* in which allatectomy has been performed, consumed very small amount of food compared to that consumed by their sham-operated controls and the operation disrupted the normal circadian feeding rhythm as well (George Abraham and Muraleedharan 1987). However, application of an anti-allatin, Precocene-II on the above larvae brought down food consumption even though with little effect on the feeding rhythm. Hence it is apparent that in *A. janata* also JH stimulated feeding. In the same larvae treated with Precocene-II, midgut protease activity (Reema Mathews and Muraleedharan 1987) and midgut carbohydrases (Raman and Muraleedharan 1987) were found to be reduced to a significant level while additional supply of JHa (ZR 515) by topical application elevated the enzyme levels. However, treatment of JHa and Precocene-II had no significant influence on the enzyme activity rhythms. In the tobacco horn worm, *Manduca sexta* larvae, starvation stimulated CA activity with the induction of supernumerary moult (Jones *et al* 1980) and according to Bhaskaran (1981) both CA and MNC are necessary for the induction of a supernumerary moult in this larva and he suggests that starvation activates brain to synthesise/or release more allatotropic hormone.

Electron microscopic studies of the midgut region in 5 different species of lepidopterans, *B. mori*, *Pieris rapae*, *Papilio xultus*, *Samia cynthia ricini*, *Lymantria dispar* and *Galleria mellonella* have demonstrated the presence of gut endocrine cells with an abundance of neuroendocrine granules which are dispersed through out the epithelia of the larval, pupal and adult midgut (Endo and Nishiitsutsuji-Uwo 1981). The hormonal principles from these cells may have a stimulatory effect on enzyme secretion as is seen in vertebrates.

3. Reproduction

Raabe (1982) opined that JH and ecdysone act during certain phases of reproduction in insects, but in these cases as in all functions neurohormones play a pivotal role and are involved in all mechanisms related to reproduction. JH is found to be both

tissue and species specific in insects (Koeppe *et al* 1985) and in the majority of insects JH is the gonadotropin regulating vitellogenesis. In addition it has been found to be involved in other reproductive phenomena like early events in gonads, control of accessory glands, reproductive behaviour etc.

3.1 *Vitellogenesis*

There remains a group of observations indicating the involvement of neuroendocrine factors in the process of vitellogenesis in some of the phytophagous insects (Girardie 1966; Minks 1967). McCafferey (1976) demonstrated that electrocoagulation of MNC from brain prevents vitellogenesis and implantation of CA into these operated insects did not result in the restoration of egg production completely even though a few follicle cells deposited yolk in locusts. Similarly in *N. cinerea* decapitation in females did not prevent egg production in the normal fashion if CA has been implanted into these (Luscher 1968). In *D. cingulatus* extirpation of MNC and their reimplantation showed that these cells stimulated vitellogenesis. But subsequent studies in allatectomised females appeared to demonstrate that CA also stimulated yolk deposition. Jalaja and Prabhu (1977) in the light of the above findings concluded that in this plant bug, CA-hormone (JH) stimulated vitellogenesis and the MNC had a trophic effect on CA activity thus indirectly stimulating vitellogenesis. CA have been found to be indispensable for yolk deposition in many other phytophagous insect species as well (Highnam *et al* 1963; Pener 1965; Strong 1965; Joly 1968). The most conclusive evidence that JH regulates vitellogenin synthesis came from the elegant *in vitro* demonstration in locusts by Wyatt *et al* (1976). They cultured fatbodies from allatectomised females in the medium containing hormonal analogues. Both CA and MNC from brain stimulated protein synthesis in the fatbodies of adult females of *D. cingulatus* (Raji Raghavan and Muraleedharan 1985). Extirpation of either the brain or CA from the female pupae of *M. sexta* hampered post emergence egg maturation in the emerging adults (Siew and Gilbert 1971). In fact vitellogenesis starts even at the pupal stages in most of the lepidopterans and allatectomy performed in pupae or adult stage does not affect the process in *Hyalophora* (Williams 1952) and in *Philosamia* (Ichikawa and Nishiitsutsuji-Uwo 1959). The exact mechanism of action of brain hormone on vitellogenic process is still unknown. Some suggest that their action on yolk deposition is through protein metabolism. But in locusts the passage of certain haemolymph proteins into the oocytes is favoured by MNC while the passage of other proteins is favoured by CA.

The ovaries and haemolymph of different kinds of reproductively competent adult females contain ecdysteroids. In *Locusta* ecdysone is being synthesised from cholesterol in the follicle cell sheath surrounding the terminal oocyte which is being controlled by a neurohormonal factor (Hoffmann *et al* 1980). In these insects ecdysone has been detected towards the end of egg maturation and just before oviposition and according to Hagedorn (1985) ovarian ecdysone can play an important role in the control of insect vitellogenesis. In many of the haematophagous insects especially in mosquitoes ovary has been found to produce a releasing factor that permits egg development neurosecretory hormone (EDNH) to be released after a blood meal (Borovsky 1982; Lea and Van Handel 1982).

3.2 *Oostatic hormone*

In many of the insect species, ovary has been found to exert a feedback control th results in an inhibition of vitellogenesis under certain conditions when ripe oocyt cannot be laid (Engelmann 1957; Nayar 1958; Adams *et al* 1968; Meola and Lea 197 Huebner and Davey 1973). Vitellogenesis inhibition is due to a substance comir from the ovary—an anti-gonadotropin—to act through pars intercerebral neurosecretory cells.

3.3 *Spermatogenesis*

Control mechanisms of spermatogenesis in insects have not been well understoo However, it has been found that in silkworms especially in *H. cecropia* and in *Sam cynthia* spermatogenesis is under the influence of ecdysone (Schmidt and Willian 1953). The regulation of spermatogonial mitosis in *Samia cynthia* (Kambysellis ar Williams 1971) and in *Locusta* (Dumser 1980) is found to be under a stimulato control of ecdysone. Also Ambika and Prabhu (1978) have demonstrated that J directly stimulated transformation of spermatocytes into spermatids and sperms.

3.4 *Oviposition*

In most cases oviposition in insects takes place after mating and the brain plays a important co-ordinating role in this process. It has been found that the mat females of *B. mori* contains a substance that induces oviposition in an unmat female (Moika 1941). Also in the bug *Iphita limbata* (Nayar 1958) and in *Schistocer gregaria* (Okelo 1971) premature egg laying occurred when the haemolymph ovipositing females was injected into the females with nearly mature egg According to Raabe (1982) the oviposition factor has been present throughout t CNS whose production and release are controlled by the cephalic nerve centres. T specific action of this factor has also been found to be on the genital valves. In t grasshopper *Melanoplus* brain extracts stimulated egg laying (Friedal and Gillo 1976).

3.5 *Accessory glands*

In most of the female orthopterans, a number of accessory sex glands and organs a associated with oocyte maturation, fertilization and encapsulation. These accesso glands include the left and right colletrial glands, the spermatheca, the vestibul organ, the dermal glands, the ovipositor, the valvula and the genital atrium. The on accessory sex gland known to be regulated by CA is the left colletrial gland and eve here the exact mechanism of its control is uncertain. In orthopterans, it has be found that JH regulates either directly or indirectly by inducing protein synthesis by stimulating protocatechuic acid accumulation and calcium oxalate cryst formation (Raabe 1982). In acridids like *Gomphocerus* JH deprivation by allatecton prevented normal fluid accumulation in accessory glands (Loher 1965). Howeve very little information is available concerning the role of JH in the development male accessory glands.

3.6 *Mating behaviour*

Several of the morphological and behavioural changes associated with the onset of mating have been found to be under the influence of JH. The experiments performed by Riddiford and Williams (1971) on the great saturniid moth, *Antherea polyfemus* and on *H. cecropia* revealed the action of corpora cardiaca and corpora allata in the production of female pheromones and in calling behaviour.

Even though a lot of information is available on the endocrine mechanisms operative in different species of insects on feeding and reproduction, a lot of gaps in respect of the exact mechanisms of action of various hormonal factors in the context of both the above processes need further ingenius and accurately designed experiments utilising the modern technological developments. Another important factor to be considered is that these regulatory mechanisms in different groups of insects seem to be different as evidenced by the available literature on the topic.

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Nutritional value of malvaceous seeds and related life-table analysis in terms of feeding and reproductive indices in the dusky cotton bug, *Oxycarenus laetus* Kirby (Hemiptera: Lygaeidae)

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Abstract. Seed host diversity and subsequent impact of the nutritive value of different malvaceous host plant seeds determined the life-history consequences and the seed-use pattern of the dusky cotton bug, *Oxycarenus laetus* Kirby. Significant differences were observed in the feeding, food utilization and reproductive success of the bugs depending upon the type of seed food utilized as well as its biochemical profile particularly proteins, nitrogen, carbohydrates, phenols and lipids. Analysis of the different life-table statistics such as developmental time, maturation time, net reproductive rate (R_o), and the intrinsic rate of increase (r_m) revealed seeds of crop plants to be better utilized when compared to malvaceous weed hosts.

Keywords. Nutritional value of seeds; life-table analysis; feeding and reproductive indices; dusky cotton bug; *Oxycarenus laetus*.

1. Introduction

Feeding and reproduction in insects are very closely related to nutritional factors, the qualitative and quantitative aspects of which have an impact not only on fecundity, but also on the rates of growth and development. Johansson (1958) observed that the quality and quantity of food influence the pre-oviposition period and fecundity in *Oncopeltus fasciatus* (Dallas). Chaplin and Chaplin (1981) studied the growth of *O. fasciatus* on the seeds of *Asclepias* spp. and attributed that the seed nitrogen content and seed cardenolide content were the important parameters accounting for 87–96% of the variability in growth dynamics of the bugs. The dusky cotton bug, *Oxycarenus laetus* Kirby is particularly associated with several malvaceous crop and weed seeds for its growth, development and reproduction (Thangavelu 1978). Observations by Ananthakrishnan *et al* (1982) showed that the diversity in different malvaceous seed hosts induced differential preference in *O. laetus* with respect to orientation and seed use pattern. A correlation of the nutritional values of different malvaceous crop and weed hosts with the life-history traits of *O. laetus* involving feeding and reproductive indices, appear essential for a better understanding of the reproductive biology of these insects.

2. Materials and methods

Adults and nymphs of *O. laetus* were maintained in the laboratory on various malvaceous host plant seeds. Fresh seeds were provided on every alternate day and moisture requirements were met by placing wads soaked in water inside the container.

2.1 *Growth, fecundity and food utilization studies*

The comparative rate of development, growth rate and fecundity rates of *O. laetus* on different malvaceous crop and weed hosts were computed as described by Ananthakrishnan *et al* (1982). To assess the impact of food on the development of ovaries, paired ovaries of females (3 days after eclosion) were dissected in insect ringer and the number of mature oocytes/ovary as well as the percentage ovarian indices (calculated as the ratio of the total ovariole length and length of the basal oocyte) were calculated. In addition, the quantity of different seed food utilized was also assessed based on the methods of Saxena (1963).

2.2 *Life-table analysis of O. laetus*

Laboratory cultures of *O. laetus* continuously reared on different malvaceous host plant seeds for atleast two generations were used for life-table parameter studies. All the experiments were carried out at 32·5°C ± 0·5°, 85% RH and 13L:11D photoperiod. When nymphs reached the 5th instar stage, the cultures were monitored daily and newly eclosed adults were removed from the culture, paired with a member of the opposite sex and 25 such pairs were so monitored. Date of laying of each batch of eggs (clutch) and the number of eggs per clutch were recorded for each female. To determine the time of development from egg to mature adult, a sample of 50 eggs from each test population was monitored separately. Time of hatching and duration from eclosion to adult emergence were recorded which when summed up yielded the average interval from birth to eclosion. This value was added to the maturation time (eclosion to first oviposition interval) for each female to give the value of absolute age. The various population statistics followed in the present study was then computed as per the methods of Dingle and Baldwin (1983).

2.3 *Biochemical analysis*

The different malvaceous host plant seeds were analysed for various biochemical components. The total carbohydrates were estimated by the methods of Dubois *et al* (1956), proteins by Lowry *et al* (1951) and phenols by Bray and Thorpe (1954). The nitrogen was analysed by using Kjeldahl's apparatus following the procedure of Vogel (1963). For the estimation of total lipid content of seeds, 5 g of the material was ground and extracted in petroleum ether for 24 h using a Soxhlet apparatus. The total quantity of lipids was then estimated by gravimetric method. To the total lipids extracted from different seeds, 10 ml of ethanol, 3 ml of 28% NH_4OH, 25 ml of petroleum ether and 25 ml of diethyl ether were added to a separating funnel, shaken for 5 min and allowed to stand for 20 min. After draining the bottom phase, the ether phase was dried to which 3 ml of 0·5 N NaOH in methanol was added and heated in a steam bath for 15 min. To this 5 ml of water was added followed by 2 N HCl. After dissolving the material, the pH was adjusted to 2. The fatty acid methyl esters were then extracted into 5 ml of petroleum ether and 5 ml of diethyl ether from the acidified methylated lipid extract. Fatty acid profiles were then analysed using a Hewlett Packard HPLC system fitted with a Hypersil ODS 5 μm column with water and acetonitrile as mobile phase at a flow rate of 0·45 μm as per the gradient

programme of Schuster (1985). The different fatty acids were identified according to their retention time which was detected at two wavelengths (210 and 230 nm).

3. Results

3.1 *Comparative growth rate, development and longevity of O. laetus*

Observations on the comparative growth rate, duration of post-embryonic development and longevity of *O. laetus* showed significant variations when fed with the seeds of different malvaceous host plants (table 1). The growth rate (GR) was maximum (29·75) on *Gossypium hirsutum* and was also significantly different from other seed diets. The GR for *Hibiscus sabdariffa* var *albus* (26·32) was on par with *H. s.* var *rubber* (25·38). Similarly, there were no significant differences in the GR among *Abelmoschus esculentus*, *Abutilon crispum* and *A. indicum*. Data on the time taken for post-embryonic development indicated that on *G. hirsutum*, *O. laetus* took only 21 days to complete the life-cycle as against other hosts, wherein a longer time was required. The longevity of adults also varied significantly when fed with the seeds of various malvaceous host plants, seeds of crop plants enabled the bugs to live longer compared to those of weed hosts. Females and males maintained on *G. hirsutum* lived for 48·50 and 26·45 days respectively. These observations revealed that seeds of malvaceous crop plants were superior compared to those of weed hosts in supporting better growth and development of *O. laetus*.

3.2 *Food utilization pattern of O. laetus*

The food utilization patterns of *O. laetus* on different malvaceous seed hosts are furnished in table 2. Computation of dry weight of food ingested and utilized by the

Table 1. Comparative growth index, duration of post-embryonic development and longevity of *O. laetus* on different malvaceous host plants.

Host plants	Growth rate	Duration of post-embryonic development (days)	Mean longevity (days)	
			Males	Females
Crops				
G. hirsutum	29·75 a	21·00 a	26·45 a	48·50 a
H. s. var *albus*	26·32 b	21·47 a	23·15 b	42·40 b
H. s. var *rubber*	25·38 b	22·00 b	23·00 b	42·10 b
A. esculentus	19·75 c	22·12 b	23·00 b	40·10 b
Weeds				
A. crispum	19·23 c	22·40 b	22·75 b	38·00 c
A. indicum	18·20 c	23·60 c	22·75 c	37·75 c
H. vitifolius	16·72 d	23·60 c	21·00 c	32·50 d
S. rhombifolia	16·30 d	23·60 c	20·00 c	32·00 d
S. rhomboidea	14·85 d	24·20 d	19·00 c	32·00 d
H. panduraeformis	14·25 d	24·54 d	19·00 c	31·50 d

Mature dry seeds were provided as diets. Values represent mean of 5 replicates.
GR: Number of 5th instars moulting into adults and duration for moulting.
Means followed by a common letter are not significantly different at 1% (DMRT).

Table 2. Food utilization of *O. laetus* on certain malvaceous host plants.

Host plants	Dry wt. of food ingested (mg/ insect/day)	Dry wt. of excreta (mg/ insect/day)	Dry wt. of food utilized (mg/insect/day)	Proportion of food utilized
G. hirsutum	*2·053 ± 0·11	0·274 ± 0·041	1·779 ± 0·391	0·863
H. s. var *albus*	1·832 ± 0·041	0·263 ± 0·001	1·569 ± 0·012	0·856
H. s. var *rubber*	1·830 ± 0·140	0·262 ± 0·012	1·568 ± 0·001	0·857
A. esculentus	1·406 ± 0·032	0·244 ± 0·011	1·161 ± 0·011	0·826
A. crispum	1·403 ± 0·011	0·242 ± 0·101	1·161 ± 0·101	0·827
A. indicum	1·404 ± 0·050	0·242 ± 0·021	1·162 ± 0·011	0·828
H. vitifolius	1·213 ± 0·132	0·241 ± 0·011	0·972 ± 0·014	0·801
S. rhombifolia	0·932 ± 0·020	0·241 ± 0·012	0·691 ± 0·013	0·741
S. rhomboidea	0·849 ± 0·020	0·219 ± 0·021	0·630 ± 0·011	0·742
H. panduraeformis	0·837 ± 0·110	0·216 ± 0·013	0·621 ± 0·112	0·742

*Represent Mean ± S.E. Dry mature seeds were provided as diets.

5th instar of *O. laetus* revealed that it was highest for *G. hirsutum* (2·053 mg/day and 1·779 mg/day respectively) as against other seed diets. Also, seeds of crop plants were better ingested and utilized by *O. laetus* when compared to those of malvaceous weed hosts. The proportion of food utilized also exhibited a similar trend wherein crop plant seeds were much more preferred compared to weed hosts. These results confirm that seeds of crop plants were of better quality than those of weed hosts.

3.3 *Impact of seed diet on the fecundity of O. laetus*

The mean number of eggs laid by *O. laetus* when fed with the seeds of different host plants are indicated in figure 1. The fecundity was highest (31·50 eggs/female) when fed with *G. hirsutum* followed by 27·50, 26·35 and 25·00 eggs/female on *H. s.* var *albus*, *H. s.* var *rubber* and *A. esculentus*. Similarly, when *G. hirsutum* was the seed diet, the fecundity index was significant and maximum (10·45) compared to other seed diets. Computation of percentage ovariole index as well as the mean number of oocyte/ovary revealed that seeds of *G. hirsutum* to be the most preferred host.

3.4 *Life-table parameters of O. laetus*

The life-table and population consequences of *O. laetus* on 3 seed hosts viz *G. hirsutum*, *A. esculentus* and *A. crispum* (weed) were studied. The total developmental time of *O. laetus* on these hosts was found to be 21, 22 and 23 days respectively.

3.4a *Survivorship*: Figure 2 depicts survivorship of l_x which is the proportion of individuals alive at birth (age 0) and still alive at age x. When reared on *G. hirsutum* the mortality of females was least upto the 18th day of their eclosion beyond which the proportion of females surviving started decreasing gradually upto 38th day. Beyond the 40th day the proportion of females surviving declined rapidly and almost all the females died by the 50th day of their eclosion. On *A. esculentus* the mortality of females was least upto the 12th day of adult eclosion and rapidly increased thereafter. On the weed host, *A. crispum* the mortality started increasing even from

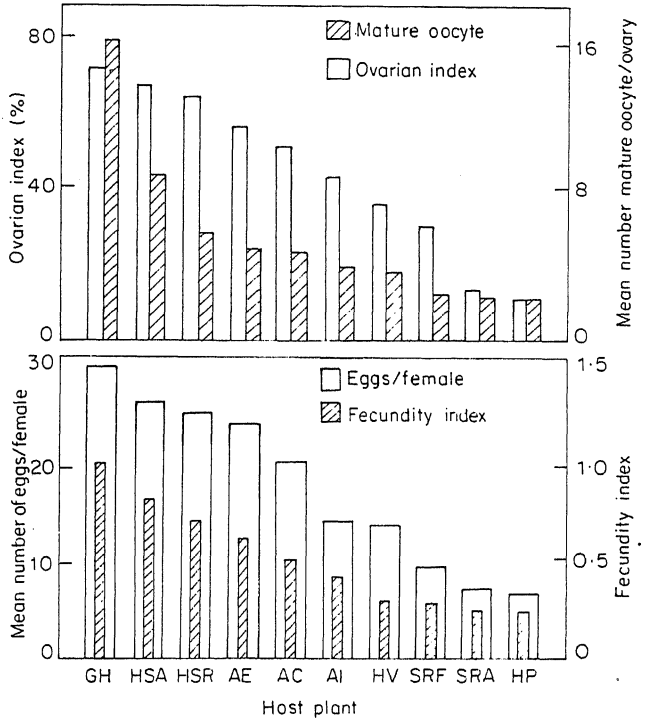

Figure 1. Impact of seed diets on the fecundity and ovarian indices of *O. laetus*. (GH, *G. hirsutum*; AE, *A. esculentus*; HSA, *H. s.* var *albus*; HSR, *H. s.* var *rubber*; HV, *H. vitifolius*; AC, *A. crispum*; AI, *A. indicum*; SRF, *S. rhombifolia*; SRA, *S. rhomboidea*; HP, *H. panduraeformis*).

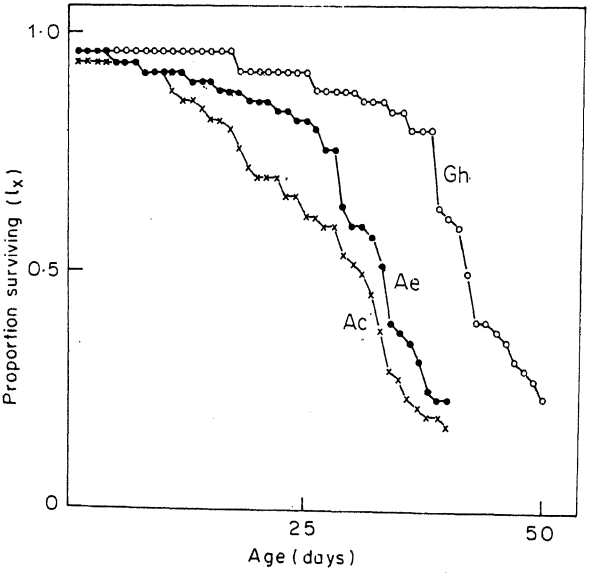

Figure 2. Survivorship curves of *O. laetus* under the influence of different seed diets. (GH, *G. hirsutum*; AE, *A. esculentus*; AC, *A. crispum*).

the 10th day. However, on *A. esculentus* and *A. crispum* all the females died by the 40th day of their eclosion.

3.4b *Reproduction*: The time course of reproduction, as well as its onset, was markedly influenced by the type of host plant seeds provided for the female. The influence of type and quality of food on the egg pattern are summarized in figure 3 which gives the egg curves produced with each type of diet. When fed with the seeds of *G. hirsutum* reproduction commenced earlier i.e. on the 8th day after adult eclosion, accelerated on the 11th and 12th day, decreased on the 13th day and reached at its peak on the 16th day with maximum egg production on the 17th day. On analysing the egg curve for *A. esculentus* it was apparent that the onset of egg laying commenced only on the 9th day. Beyond the 30th day the number of eggs/female decreased considerably upto the 40th day, after which no individuals were alive. Rearing on the seeds of the weed host, *A. crispum* the egg laying started only on the 11th day of eclosion of female which gradually increased and beyond the 18th day the number of eggs produced sharply declined. Thus, a comparative assessment of the egg curve indicated that the birth rate in female in terms of the number of eggs/female to be maximum for *G. hirsutum* compared to *A. esculentus* and *A. crispum*.

3.4c *Rate of increase* (r_m): The influence of different diets on reproduction was

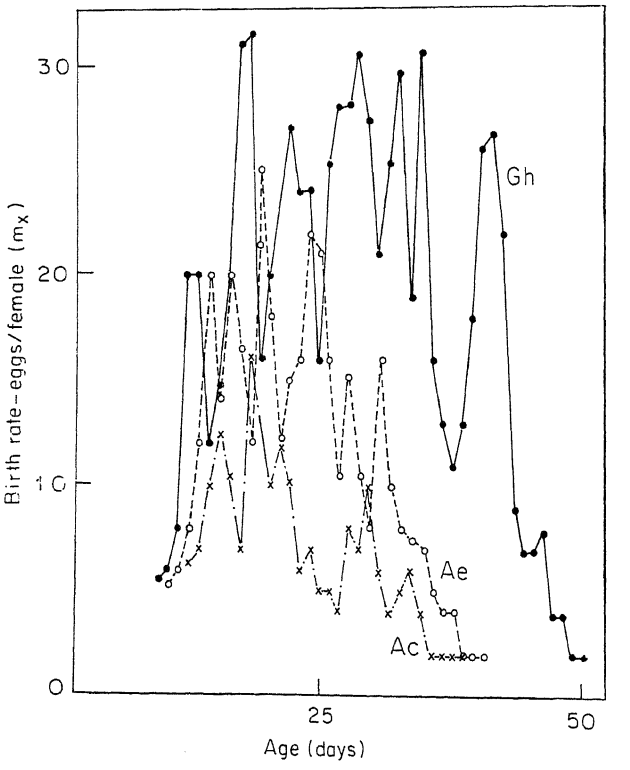

Figure 3. Birth rate of *O. laetus* under the influence of different seed diets. (GH, *G. hirsutum*; AE, *A. esculentus*; AC, *A. crispum*).

determined by computation of the population rate of increase. The r_m measure denote the intrinsic rate of increase of population with a stable age distribution. Feeding with cotton seeds gave r_m value of 0·1448 followed by 0·1365 and 0·1229 for *A. esculentus* and *A. crispum* (table 3). Similarly, the net reproductive rate (R_o) representing the total female births were 640·14, 292·35 and 143·05 respectively on these host plants. The different life-table statistics computed for *O. laetus* are represented in table 3.

3.5 *Nutritive value of malvaceous seeds*

The results of the various biochemical parameters analysed are indicated in table 4. Analysis of the total proteins revealed that it was maximum (236·80 mg/g) on *G. hirsutum* as against 114 and 107·50 mg/g obtained for *H. s. var albus* and *H. s. var rubber*. The total protein content of weed hosts were very low when compared to crop plants. The estimation of carbohydrates for different seed hosts did not reveal marked variations among different host plants. However, it was noted that seeds of

Table 3. Life-table statistics of *O. laetus* on malvaceous host plants.

Parameters	*G. hirsutum*	*A. esculentus*	*A. crispum*
Developmental time	21·00 days	22·00 days	23·00 days
Maturation time	7·00 days	8·00 days	10·00 days
Time to death	49·00 days	39·00 days	37·00 days
Clutch size	3·50	2·70	1·50
Interclutch interval	1·50 days	2·00 days	2·50 days
Number of clutches	41·00 days	31·00 days	28·00 days
Age at first reproduction (α)	28·00 days	30·00 days	33·00 days
Mean number of eggs/female (m_x)	31·50	25·00	21·00
Net reproductive rate (R_o)	640·14	292·35	143·05
Mean generation time (T)	44·61	41·59	40·36
Intrinsic rate of increase (r_m)	0·1448	0·1365	0·1229
Capacity for increase (r_c)	0·1657	0·1384	0·1296
Cohort generation time (T_c)	38·99 days	41·03	40·38

Table 4. Chemical composition of different malvaceous host plant seeds.

Host plants	Proteins (mg/g dry wt.)	Carbohydrates (mg/g dry wt.)	Phenols (mg/g dry wt.)	Nitrogen (%)	Lipids (%)	C/N ratio
G. hirsutum	236·80 ± 0·13[a]	12·00 ± 0·59	18·75 ± 0·11	8·73 ± 0·63	41·20 ± 0·11	1·37
H. s. var *albus*	114·00 ± 0·23	15·00 ± 0·10	5·25 ± 0·20	5·14 ± 0·10	21·08 ± 0·20	2·92
H. s. var *rubber*	107·50 ± 0·11	18·00 ± 0·23	4·75 ± 0·10	5·36 ± 0·15	24·52 ± 0·23	3·36
A. esculentus	142·50 ± 0·33	16·00 ± 0·10	4·25 ± 0·04	5·62 ± 0·22	14·05 ± 0·11	2·85
H. ritifolius	61·00 ± 0·10	19·00 ± 0·10	1·75 ± 0·20	4·64 ± 0·11	4·50 ± 0·20	4·09
A. crispum	60·00 ± 0·89	21·00 ± 0·13	3·25 ± 0·11	4·94 ± 0·56	4·00 ± 0·10	4·25
A. indicum	52·80 ± 0·11	23·50 ± 0·11	3·05 ± 0·03	4·73 ± 0·26	4·15 ± 0·03	6·03
S. rhombifolia	49·80 ± 1·41	21·10 ± 0·13	2·50 ± 0·10	4·36 ± 0·03	2·15 ± 0·03	5·16
S. rhomboidea	33·60 ± 0·33	22·50 ± 0·21	2·75 ± 0·13	4·10 ± 0·11	2·10 ± 0·13	5·49
H. pandurae formis	23·60 ± 0·23	20·00 ± 0·12	3·50 ± 0·10	3·05 ± 0·47	2·00 ± 0·13	6·58

[a]Represent Mean of 3 replications ± SE.

G. hirsutum were low in total carbohydrate content as against other host plants. But the total nitrogen (8·73%) and phenolic (18·73 mg/g) contents were relatively high. Estimation of total lipids also indicated that it was higher for seeds of crop plants when compared to weed hosts. The carbohydrate/nitrogen ratio, a measure to indicate the degrée of susceptibility of host plants to insect attack, were lower for crop plants as against weed hosts indicating that seeds of crops were more preferred by *O. laetus.*

Figure 4 provides the HPLC analysis of the fatty acid methylester profiles of some important malvaceous crop and weed seeds utilized by *O. laetus.* The individual fatty acids were identified based on the retention time and the percentage area composition of the relative compounds were obtained (table 5). It can be seen that the seeds of *G. hirsutum* contained arachidic, stearic, oleic, palmitic, linoleic and linolenic acids. Linolenic acid was not detected in the seeds of *A. esculentus* and *H. s.* var *albus.* Similarly, linolenic and arachidic acids were not identified in the seeds of *H. s.* var *rubber.* Interestingly, seeds of the weed hosts, *Sida rhombifolia* and *S. rhomboidea* were deficient in most of the important fatty acids. These observations confirm the fact that seeds of crop plants are richer in several fatty acids as against those of weed plants.

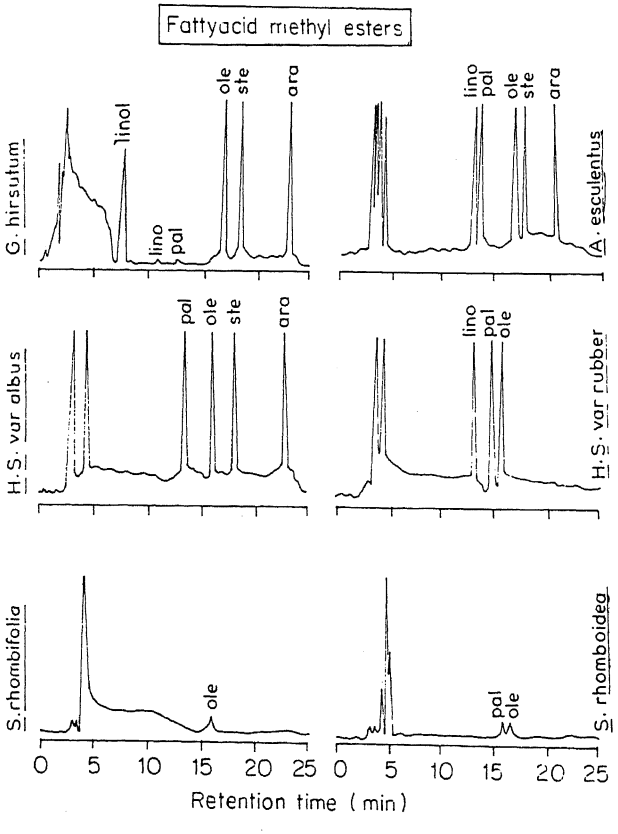

Figure 4. HPLC profile of triglyceride methyl esters in different malvaceous seeds.

Table 5. Qualitative profile of triglyceride methyl esters in different malvaceous seeds.

Host plants	Retention time (min)	Area(%)	Host plants	Retention time (min)	Area (%)
G. hirsutum	1·04	0·038	*H. s.* var *albus*	3·71	97·623
	1·33	0·050		3·82	1·558
	1·54	0·020		12·87	0·030
	1·77	0·205		14·18	0·154
	7·24	0·398		15·16	0·666
	7·71	0·033	*H. s.* var *rubber*	2·99	16·503
	7·83	0·130		4·40	64·581
	10·05	0·022		13·07	7·892
	10·12	0·014		15·61	2·603
	12·08	0·074		17·34	2·645
	15·96	3·775		22·55	5·775
	17·80	74·501	*S. rhombifolia*	2·71	0·722
	22·47	20·740		2·88	3·742
A. esculentus	2·65	0·300		3·90	94·267
	2·96	1·942		15·45	1·269
	3·05	5·961	*S. rhomboidea*	2·81	0·675
	3·54	3·035		2·97	5·825
	3·94	69·857		4·05	30·860
	12·39	10·164		4·35	22·196
	12·64	2·283		4·51	39·907
	12·71	2·840		15·35	0·269
	15·83	1·210		15·70	0·267
	16·64	0·933			
	16·97	0·243			
	17·04	1·208			
	19·59	0·025			

4. Discussion

Feeding and reproduction in insects are two closely linked physiological processes, the magnitude of which is strongly influenced by nutritional factors. Seeds, the prime diet of many lygaeid bugs, are also highly nutritious. Species of the genus *Oxycarenus* are primarily restricted to the plant order Malvales which include the families Malvaceae, Sterculiaceae and Bombaceae (Adu-Mensah and Kumar 1977). In India, the dusky cotton bug, *O. laetus* utilizes mature and dry seeds of several crop and weed hosts (Thangavelu 1978; Ananthakrishnan *et al* 1982) and in the present study it was found that seed host diversity had a profound bearing on the growth, development and longevity of the bugs. The type of seed food is known to influence the survival, growth and development of several other species of heteropterans also, such as the lygaeids, *Lygaeus equestris* (L) (Kugelberg 1973) and *O. fasciatus* (Ralph 1976), pentatomid *Piezodorus guildnii* (Westwood) (Panizzi and Slansky 1985), and mirid *Cyrtopeltis tenuis* Reut. (Raman and Sanjayan 1984). The present investigation also showed that seeds of malvaceous crop plants favoured faster development of *O. laetus* thereby attributing the fact that growth rate and growth efficiency increases as the suitability of diet increases (Chaplin and Chaplin 1981). On seeds of weed hosts the nymphs exhibited a slower growth rate and consequently the moulting period was also delayed which indicate that they have to spend additional time in

order to attain the critical body size that would eventually lead to adult ecdysis (Blakley and Goodner 1978).

The utilization of a host plant by an insect is determined not only by its ability to locate and colonize on the prospective host plant, but also on the capacity to ingest, assimilate and convert the food into body tissues (Slansky 1982). The food utilization pattern of *O. laetus* indicated that the quantity of food ingested and utilized were maximum when maintained on the seeds of *G. hirsutum* and other crop plants compared to weed hosts. The differences in the amount of food ingested and utilized from different malvaceous seed hosts also profoundly influenced the reproductive performance of adult females. It has also been shown that maximum egg production occurred when the bugs were maintained continuously on a diet of cotton seeds only and any interruption in its dietary schedule, either by maintaining on water alone or by substituting seeds of lower nutritive value, significantly affected the total egg out-put (Raman and Sanjayan 1983). The natural food has been found to influence the reproduction in seed-feeding heteropterans in several ways. For instance, in *L. equestris* (Kugelberg 1973) and *O. fasciatus* (Ralph 1976) the length of oviposition period, number of eggs laid and longevity of adults were affected by type of seed food. Similarly, Ewete and Osisanya (1985) showed that seeds of cotton, okra and kenaf profoundly influenced the development, longevity and fecundity of *Oxycarenus gossypinus* Dist. in Nigeria. In addition, inadequate diet or diet of inferior quality during 5th stadium irreversibly affected growth, development, reproduction and flight capacity in the pentatomid *Nezara viridula* (F) (Kester and Smith 1984). Computation of age-specific fecundity life-table of *O. laetus* revealed that the type of seed food influenced the time course of reproduction as well as its onset. When fed with the seeds of *G. hirsutum*, egg-laying commenced earlier and accelerated rapidly leading to maximum egg out-put. Higher intrinsic rate of increase and net reproductive rates were computed for *G. hirsutum* compared to *A. esculentus* and *A. crispum*. Similar observations were also reported in the case of the milkweed bug *O. fasciatus* (Klausner *et al* 1980), pentatomid *P. guildnii* (Panizzi and Slansky 1985), and coried *Clavigrella tomentosicollis* (Stall) (Iheagwam 1982) wherein higher reproductive rate, intrinsic rate of increase and net reproductive rates were consistently observed when fed with the seeds of preferred host.

The quality of host plant is known to influence the feeding behaviour of phytophagous insects and each species requires a particular quantitative composition of nutrients in its diet to complete normal growth and development. Interestingly, in the present study a strong relationship was found to exist between the food preference of *O. laetus* and biochemical composition of host plant seeds. Even though all the seed hosts belong to the family Malvaceae, it was observed that the food utilization varied among different seeds, presumably due to the differences in their nutritional composition. Seeds of *G. hirsutum*, the most preferred host, contained higher total nitrogen, proteins, lipids and lower carbohydrates and narrow C/N ratio. It was stressed that increased nitrogen content increases fecundity, longevity and survival rates of phytophagous insects (McNeill and Southwood 1978; Chaplin and Chaplin 1981; Ananthakrishnan *et al* 1982; Raman and Sanjayan 1984) while low sugars coupled with high protein content (Beck 1956) favoured greater host preference. In the present investigation increased phenolic contents were associated with greater preference of *O. laetus* probably indicating that their concentrations were not substantially high in order to have an inhibitory effect on

feeding. Seed host preference was also enhanced by increased total lipid content and the presence of several fatty acid methyl esters in crop plants. Free fatty acids (non-volatile lipids) are also reported to act as biting or feeding stimulants for certain insects such as the silkworm *Bombyx mori* (linolenic acid in mulberry leaves); the fire ants *Solenopsis* sp. (linoleic and linolenic acids) and in the *Dermestes* beetles (palmitic and stearic acids) (Seigler 1983). Also, in *O. fasciatus* the body lipid requirements were found to be met by the unsaturated fatty acids present in the milkweed seeds and the fatty acid composition of the bugs was influenced by the type of seed diet (Nation and Bowers 1982). In general, it appears that the nutritional quality of the diet accounts for the major portion of the variation in rates and efficiency of food utilization leading to increased reproductive performance (Scriber and Slansky 1981). Hence, the increased food utilization favouring faster developmental rates and higher fecundity values of *O. laetus* when fed with seeds of crop plants could be attributed to the nutritional superiority of the seed food.

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Influence of leaf age on feeding and reproduction in *Raphidopalpa atripennis* F. (Coleoptera: Chrysomelidae)

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Abstract. The influence of leaf age on the quantitative food intake and the reproductive success of the cucurbit specialist *Raphidopalpa atripennis* F. is provided. The host plant influence on the adult longevity and survival percentage were also studied. The differential feeding behaviour and the relative preference of this beetle towards mature leaves and better reproductive success are attributed to the chemical composition of the host leaf. The individual fatty acid profile of the various hosts as well as the different leaf ages is also attempted.

Keywords. Food utilisation; leaf age; fecundity; longevity; *Raphidopalpa atripennis.*

1. Introduction

Studies on the impact of age dependent changes in foliage on feeding and reproduction in insects have been initiated in such insects as *Leptinotarsa decemlineata*, *Haltica lythri* and *Plagiodera versicolora* on individual host plant (Grison 1957, 1958; de Wilde *et al* 1969; Phillips 1976; Raupp and Denno 1980) not to mention of isolated instances in several other insects. However, information pertaining to the relative preference, comparative food utilization on different host plants and the consequent changes in the reproductive ability aspects deserve closer scrutiny. Observations on these aspects are discussed here with reference to the cucurbit infesting Chrysomelid beetle *Raphidopalpa atripennis* F.

2. Material and methods

Adults of both sexes of *R. atripennis* collected from such hosts as *Luffa acutangula* Roeb., *Luffa cylindrica* Roem. and *Mukia scabrella* Arn. were reared in the laboratory in plastic containers with their respective host leaves. Moisture requirements were met by placing a wet cotton wad in the containers. Fresh leaves were provided every alternate day.

2.1 *Quantitative food utilisation*

In order to study the quantitative food utilization by *R. atripennis* leaf discs (7·5 cm dia) or entire leaves were placed inside a petri dish over a moist filter paper of the same dimension. The test insects were fed with water for 2–3 h by providing a moist cotton to clear their guts before the start of the experiment. The initial weight of the leaf disc, the filter paper and the test insect were determined. A pair of beetles were released into the dish and covered by another petri plate. A control was also maintained without the test insect, the leaf discs, the filter paper and the test insects were

removed and reweighed. Before reweighing the filter paper and the sides of the petridishes, the left over leaf discs were carefully wiped off with a camel hair brush to collect excrements, if any. After correcting for the weight loss due to transpiration by the leaf, the comparative food utilisation on different host plants was assessed by determining the quantity of food ingested/24 h and the consumption index, growth rate, approximate digestibility, efficiency of conversion etc were calculated (Waldbauer 1968). Three replicates were made.

2.2 *Fecundity studies*

To study the fecundity of *R. atripennis* on different cucurbitaceous host leaves of different ages (young 3 day old, mature 5–10 day old, senescent—more than 10 days and flowers 3 day old) freshly hatched adult (imago) male and female pairs, whose larvae were formerly reared on their respective host roots were confined in plastic vials. Leaves of the respective host plants were provided inside the vials. The fecundity was calculated as the total number of eggs laid during the life time of an individual.

2.3 *Biochemical analysis*

Total nitrogen (Humphries 1956); total carbohydrates (Dubois *et al* 1956); total phenols (Bray and Thrope 1954); total chlorophyll (Yoshida *et al* 1976); total amino-acids (Moore and Stein 1948); and total lipids (Folch *et al* 1957) of the various cucurbitaceous host plants as well as the different leaf stages were analysed.

In order to analyse the qualitative fatty acid profile of the host leaves of different ages the total lipids extracted from the leaf tissue was added with 10 ml of ethanol, 3 ml of 28% ammonium hydroxide, 25 ml of petroleum ether and 25 ml of diethyl ether in a separating funnal, shaken for 5 min and allowed to stand for 20 min. The bottom layer was drained off, the ether fraction was dried to which 3 ml of 0·5 N NaOH in methanol was added and heated in a steam bath for 15 min. To this 5 ml of distilled water was then added and 2 N HCl were added slowly until the pH reached approximately two. The fatty acid methyl esters were then extracted into 5 ml of petroleum ether and 5 ml of diethyl ether from the acidified methylated lipid extract. Fatty acids were analysed by a Hewlett Packard HPLC system at 230 nm using an Hypersil ODS 5 μm column with water and acetonitrile as solvents at a flow rate of 0·45 ml according to the gradient programme as per Schuster (1985). The retention time and area percentage of fatty acid methyl esters were recorded.

3. Results

3.1 *Quantitative food utilization*

The amount of food ingested by *R. atripennis* varied among the different cucurbitaceous hosts. Data on the comparative food utilization of *R. atripennis* is provided in table 1. Among the 3 host plants tested the maximum amount of food ingested was on *L. acutangula* (0·0501 ± 0·003 mg), followed by *L. cylindrica* (0·0474 ± 0·003 mg)

Table 1. Quantitative food utilization in *R. atripennis.*

Host	Amount of food ingested (mg)	CI	GR	AD (%)	ECD (%)	ECI (%)
L. acutangula						
Young	0·0489 ± 0·002	2·445 ± 0·10	1·22 ± 0·35	77·50 ± 6·86	50·62 ± 4·89	49·89 ± 3·5
Mature	0·0501 ± 0·003	2·505 ± 0·015	1·34 ± 0·20	97·80 ± 7·24	54·69 ± 0·57	53·49 ± 1·33
Senescent	0·0298 ± 0·002	1·490 ± 0·10	0·68 ± 0·10	66·44 ± 3·86	47·22 ± 3·10	45·63 ± 1·00
Flowers	0·0492 ± 0·003	2·46 ± 0·015	1·17 ± 0·05	85·77 ± 5·08	55·45 ± 3·25	47·56 ± 3·33
L. cylindrica						
Young	0·0398 ± 0·000	1·990 ± 0·00	0·82 ± 0·05	94·48 ± 8·29	86·70 ± 0·16	40·95 ± 0·00
Mature	0·0474 ± 0·003	2·37 ± 0·15	1·13 ± 0·10	97·89 ± 11·23	48·70 ± 0·65	47·60 ± 9·52
Senescent	0·0197 ± 0·008	0·985 ± 0·40	0·43 ± 0·20	78·68 ± 7·24	55·48 ± 0·18	45·18 ± 5·00
Flowers	0·0419 ± 0·012	2·095 ± 0·60	1·12 ± 0·10	83·29 ± 8·21	68·78 ± 1·10	53·46 ± 16·66
M. scabrella						
Young	0·0187 ± 0·019	0·935 ± 0·50	0·49 ± 0·12	57·21 ± 4·36	91·58 ± 4·21	52·41 ± 5·20
Mature	0·0202 ± 0·002	1·010 ± 0·10	1·50 ± 0·26	90·09 ± 6·02	55·49 ± 1·02	50·00 ± 1·00
Senescent	0·0109 ± 0·004	0·545 ± 0·20	0·29 ± 0·24	82·56 ± 7·24	64·44 ± 1·08	53·21 ± 1·50
Flowers	0·0192 ± 0·008	0·960 ± 0·40	0·49 ± 1·02	85·41 ± 3·14	59·75 ± 0·86	59·75 ± 3·75

± Standard deviation

wherein the highest consumption was on the mature leaves. The food ingested was least on *M. scabrella* (0·0202 ± 0·002), the order preference being *L. acutangula* > *L. cylindrica* > *M. scabrella.* The various growth parameters computed viz CI, GR, AD, ECD and ECI were also in accordance with food preference but with some exceptions. The consumption index, growth rate and the approximate digestibility were relatively higher in the case of mature leaves and flowers compared to young and senescent leaves of all the hosts. The ECD was maximum only in the young leaves of *L. cylindrica* (86·70 ± 0·16) and *M. scabrella* (91·58 ± 4·21) than the mature leaves. With regard to ECI of flowers, *L. cylindrica* (53·46 ± 16·66) and *M. scabrella* (59·75 ± 3·75) showed a higher percentage than any other plant part.

3.2 *Feeding preferences*

The feeding preferences of *R. atripennis* was tested in a no-choice as well as free-choice situation. Among the 3 host plants offered, *R. atripennis* showed a selective preference towards the mature leaves of *L. acutangula* in a free-choice situation. But in a no-choice situation, when only *M. scabrella* was offered the beetles readily fed on them. In a condition where *L. cylindrica* and *L. acutangula* were offered the preference was towards *L. acutangula*, but when *L. cylindrica* and *M. scabrella* were offered the preference was towards *L. cylindrica.*

R. atripennis utilizes the weed plant *Mukia scabrella* only when no other cucurbit crop is available in that vicinity. If all the host plants are present in the same area only *L. acutangula* is utilised. The random search of an adult beetle guided by olfactory and visual cues result in the arrival on a suitable cucurbit host plant, following which the insect searches for a proper feeding site, depending on the age of the leaf. It is followed by antennal palpation and labial exploration where the sensillae on these areas play a vital role in the host selection process. Having located a

suitable feeding site, the beetle makes a test bite which results either in a continuous feeding till satiation or in a change of feeding site.

3.3 *Host plant influence on the longevity and survival*

The longevity of adults of *R. atripennis* as well as the survival percentage was also influenced by the host plant quality. Adults reared on the mature leaves of *L. acutangula* had a longevity of 114 ± 2·2 days followed by young leaves (106 ± 1·2 days), flowers (92 ± 3·8) and senescent leaves (80 ± 2·4 days). *L. cylindrica* ranked second followed by *M. scabrella*. With regard to the order of superiority among the different ages the same trend was followed as in the case of *L. acutangula*. The percentage survival was also maximum in the case of mature leaves of *L. acutangula* (98·6%), the minimum being 72·14% in the case of senescent leaves of *M. scabrella* (table 2).

3.4 *Host plant influence on the fecundity of R. atripennis*

Figure 1 provides the comparative fecundity of *R. atripennis* when fed on the leaves of varying ages and flowers of the 3 host plants mentioned above. The fecundity of *R. atripennis* and the total number of egg clutches showed a marked variation among the different cucurbit plants and among the leaves of various ages. The maximum fecundity was observed in the case of *L. acutangula* (218) when fed on the mature leaves. Individuals fed on young leaves and flowers had a comparatively lesser fecundity of 175 and 78 respectively than the senescent leaves. The number of egg clutches was maximum when fed on the mature leaves while the senescent leaves of all the hosts had a marked influence on the beetle resulting in a very low fecundity. As regards the fecundity of *R. atripennis* the maximum egg output was on *L. acutangula* followed by *L. cylindrica* and *M. scabrella*.

Table 2. Impact of host plant age on the longevity and survival of *R. atripennis*.

Host plant	Longevity of adults	Survival (%)
L. acutangula		
Young	106 ± 1·2	96·00
Mature	114 ± 2·2	98·60
Flowers	92 ± 3·8	83·20
Senescent	80 ± 2·4	76·80
M. scabrella		
Young	86 ± 1·3	82·28
Mature	96 ± 0·0	86·02
Flowers	79 ± 3·1	76·32
Senescent	69 ± 2·8	72·14
L. cylindrica		
Young	98 ± 3·2	91·22
Mature	112 ± 2·8	96·00
Flowers	88 ± 1·3	81·32
Senescent	76 ± 2·7	75·00

Mean of 5 replicates.
± Standard deviation.

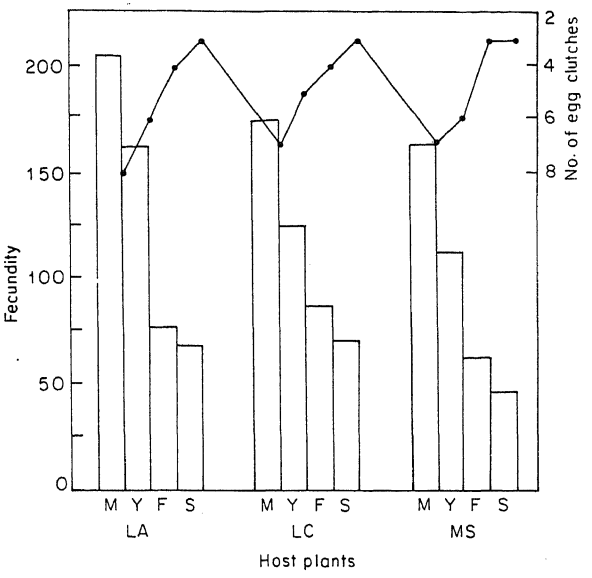

Figure 1. Impact of different cucurbitaceous host plants as well as different ages of leaves on the fecundity of *R. atripennis*. LC, *L. cylindrica*; LA, *L. acutangula*; MS, *M. scabrella*; M, Mature; Y, Young; S, Senescent; F, Flowers.

3.5 *Biochemical composition*

The chlorophyll content varied considerably among the cucurbit hosts as well as among the different leaf tissues. It was higher in the case of mature leaves of *M. scabrella* (3·60 mg/g), followed by *L. cylindrica* (2·55 mg/g) and *L. acutangula* (1·56 mg/g). The quantitative analysis of nitrogen, protein and carbohydrates showed a uniform picture in all the hosts as well as on different stages, the trend being *L. cylindrica* > *L. acutangula* > *M. scabrella*. The highest phenolic content was estimated in the case of *L. cylindrica* and the lowest in the case of *M. scabrella*, the moderate being in *L. acutangula*. An increasing trend in the total amino acid content and total phenolic content was observed in the mature leaves of all the hosts as compared to other stages. However, it was very high in *L. acutangula* than the other two hosts (table 3).

The HPLC analysis of the fatty acid composition also indicated some interesting results. The triglycerides of varying retention time with varied area percentage of all the host plants with 3 different leaf stages were provided as given in table 4. Mature leaves of *L. acutangula* showed the presence of linolenic, linoleic, palmitic and oleic acids whereas young and mature leaves showed only the presence of palmetic acid. In the case of *L. cylindrica* young leaves had palmitic and oleic acid. The pattern was more or less similar in the case of *M. scabrella* (figure 2).

4. Discussion

Phytophagous insects can be highly selective with respect to the plant they consume and that both the degree of selectivity and the identity of preferred plants can change

Table 3. Chemical composition of some cucurbitaceous hosts.

Host	Plant part	Moisture (%)	Nitrogen (%)	Chlorophyll (mg/g)	Proteins (%)	Carbohydrates (mg/g)	Phenols (mg/g)	Amino acid (mg/g)	Lipid (mg/g)	C/N ratio
L. acutangula	Young	78·14	2·17	1·73	13·56	1·63	2·00	1·80	12·5	0·75
	Mature	73·75	2·94	1·56	18·38	1·13	3·70	10·00	15·0	0·38
	Senescent	66·00	2·01	1·91	18·81	0·85	3·40	5·5	8·6	0·28
	Flowers	79·75	4·69	0·98	29·31	1·73	1·56	4·32	7·8	0·37
L. cylindrica	Young	83·28	5·32	1·73	33·25	2·38	3·00	0·6	8·2	0·45
	Mature	77·76	6·44	2·55	40·25	1·25	3·00	2·8	9·2	0·19
	Senescent	62·73	4·79	1·54	29·94	1·08	4·30	2·2	4·3	0·23
	Flowers	80·43	5·95	1·02	37·19	2·13	2·60	1·8	5·2	0·36
M. scabrella	Young	84·00	1·68	2·56	10·50	1·25	1·50	1·10	6·8	0·74
	Mature	78·77	1·75	3·60	10·94	0·95	1·60	1·50	7·3	0·54
	Senescent	76·63	1·96	2·63	12·25	0·93	1·55	1·00	5·5	0·47
	Flowers	82·50	1·12	0·42	7·00	1·10	2·15	0·82	4·2	0·98

Table 4. Showing the retention time and area (%) of the fatty acid methyl esters.

Host plant	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)
L. acutangula	*Young*		*Mature*		*Senescent*	
	2·76	0·342	2·65	0·454	2·81	8·884
	2·94	3·496	2·84	24·947	3·54	23·174
	3·31	10·837	6·42	21·926	3·75	65·014
	3·55	82·227	7·28	19·312	5·16	0·944
	4·61	0·033	9·59	24·209	5·80	1·065
	4·77	0·066	10·90	3·280	7·91	0·628
	5·09	0·280	11·54	5·568	15·63	0·293
	5·26	0·160	15·84	0·144		
	6·72	0·309	16·15	0·160		
	7·38	1·029				
	15·66	0·897				
	15·81	0·324				
L. cylindrica	2·82	0·675	2·84	1·524	2·93	6·180
	3·00	11·324	3·00	15·048	3·32	16·779
	4·74	16·603	4·39	6·854	3·44	76·440
	4·89	70·951	4·58	29·379		
	15·10	0·065	4·77	46·843		
	15·82	0·251	15·55	0·221		
	16·15	0·132	15·87	0·131		
M. scabrella	2·62	2·573	2·85	22·512	2·94	19·408
	2·71	7·285	4·25	27·217	4·21	25·004
	3·14	15·247	4·43	47·200	4·47	51·932
	3·23	74·644	5·61	0·166	6·62	0·163
	15·89	0·251	8·36	1·466	7·76	0·124
			15·86	1·060	8·10	0·053
			16·02	0·379	15·62	1·347
					15·79	0·678
					15·92	0·791

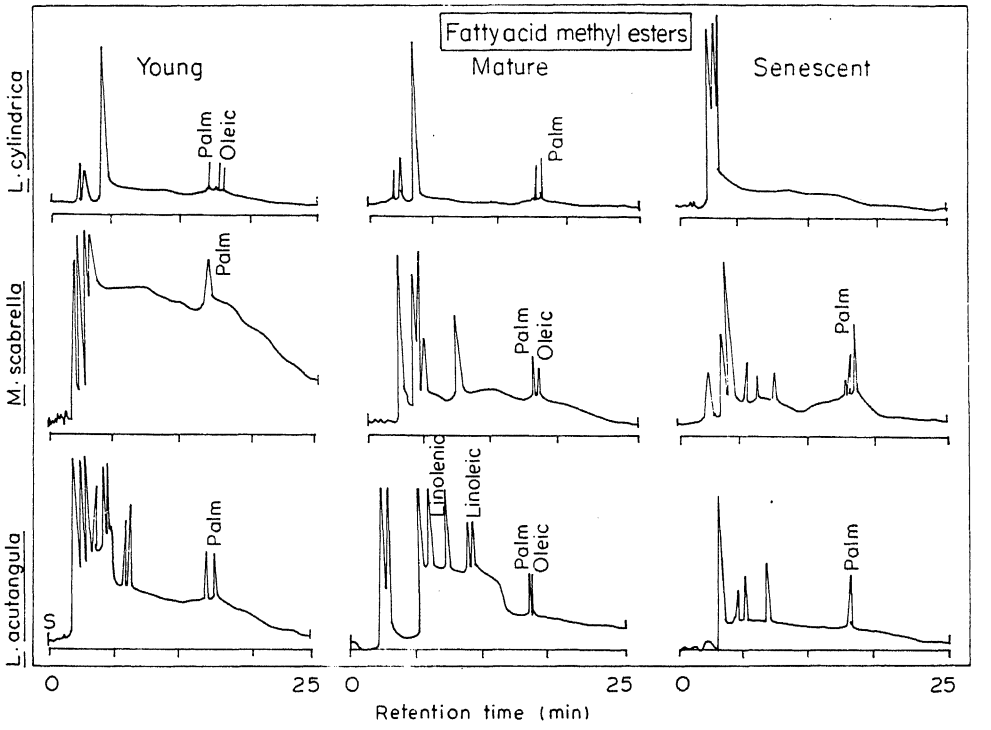

Figure 2. HPLC analyses of fatty acid methyl esters of different cucurbitaceous hosts.

during an individual's life time. It is widely recognised that plant species and individuals can differ in quality or suitability for insect growth, survival and reproduction. Hence, the mere establishment of an insect on a particular plant will not provide a clear picture with regard to the suitability of the plant. But an understanding of the efficiency of utilisation of plant resources in terms of quantitative aspects, evidenced by the biochemical composition of the hosts and the reproductive success provide an incisive picture in any investigation relating to insect-plant interactions. Hsiao and Fraenkel (1968) indicated that the host selection of the potato beetle, *L. decemlineata* is determined not only by the availability of adequate feeding stimuli and nutrient of the plant, but also by the presence of host specific substances which releases the initial feeding behaviour. While the relative preference of *R. atripennis* to the mature leaves of *L. acutangula* and a relatively higher CI, GR and AD could be attributed to the presence of such host specific substances apart from other nutrient substances, the possibility of the presence of such a feeding stimulant in this host was ruled out at least for the beetle, *Henosepilachna septima* (Mary Saroja 1982). However, higher food intake of *H. septima* on the host *L. acutangula* was suspected to be due to nutritional deficiency in the host plant which forced the beetles to consume more food to fulfil the nutritional requirements (Ganga *et al* 1985). But the present investigation revealed higher consumption rate and fecundity in *R. atripennis* when fed with *L. acutangula* which indicate the fact that this host plant is not nutritionally deficient as envisaged by Ganga *et al* (1985).

The growth rate, survival and fecundity of insects are influenced by the concentration of nutrients, particularly nitrogen and proteins in their food (Watson 1970; Mattson 1980; Scriber and Slansky 1981). In the present study biochemical analysis of the different plant parts of cucurbitaceous hosts revealed mature leaves of *L. acutangula* to contain increased amounts of nitrogen, proteins, amino acids, carbohydrates, phenols and lipids. In addition, the analysis of the fatty acid methyl ester profile of leaves of different age groups revealed the presence of linolenic, linoleic, palmitic and oleic acids in mature leaves. While senescent leaves of *L. cylindrica* did not show the presence of these fatty acids, *M. scabrella* and *L. acutangula* possessed only palmitic acid on which the food utilization as well as egg output were comparatively lesser. For instance, in the red pumpkin beetle, *Raphidopalpa foveicollis*, a definite correlation was found to exist between the utilization of different plant parts of various cucurbitaceous hosts and the level of nitrogen in those parts (Raman and Annadurai 1985). The differential feeding behaviour and increased reproductive rate of *R. atripennis* on different parts of the cucurbitaceous hosts appear not only to the variation of several nutrient substances but also due to the presence of certain secondary plant substances, notably cucurbitacin E which is presumed to be acting as a feeding stimulant (Sinha and Krishna 1970). The age of leaf is also known to influence the feeding pattern in several other insects. In *Plagiodera versicolora* higher fecundity was evident when fed with young leaves than old leaves though the females tend to lay their eggs on older leaves (Raupp and Denno 1980). On the contrary, the Mexican bean beetle, *Epilachna varivestis* preferred mature leaves wherein the fecundity was also higher compared to young or senescent leaves (Kitayama *et al* 1979). Similarly, higher concentrations of soluble nutrients in the mature leaves of eucalyptus increased the feeding preference of the psyllid, *Cardiaspina densidextra* (White 1970). Thus, the results from the present study indicate that the differential preference of the beetle, *R. artipennis* vary on leaves of different age groups which by and large appear to be due to the variations in the nutritional quality of the concerned plant part.

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Effect of food quality on fecundity of *Mylabris pustulata* (Coleoptera: Meloidae)

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Abstract. Observations on the fecundity of *Mylabris pustulata* maintained on two different food plants *Ipomoea cornea* and *Ipomoea tuberosa*, with males and females reared separately on the flowers of these two host plants are discussed. *Mylabris pustulata* reared on *Ipomoea cornea* laid 144 ± 30·80 eggs while that on *Ipomoea tuberosa* only 93 ± 38·87 eggs. The chemical composition of the flowers of *I. cornea* and *I. tuberosa* revealed that protein concentration is greater in *Ipomoea cornea* (4·88%) than *Ipomoea tuberosa* (4·64%). The quantity of food ingested is also greater when reared on *Ipomoea cornea* (1055 mg). In the light of these observations it is presumed that the food rich in protein concentrations is among the principal factors influencing fecundity.

Keywords. *Mylabris pustulata*; food quality; fecundity.

1. Introduction

Polyphagous insects feed on a variety of food plants which differ in the possession of organic and inorganic constituents. The egg production in phytophagous insects is related with the quality and quantity of food ingested (Mulkern and Brusven 1962; McCaffery 1975). Wigglesworth (1965) has stated that nutritionally poor plants will have an impact on the fecundity in phytophagous insects. Water and nitrogen contents in the food plants influence the egg production in insects (Kehat and Wyndham 1972; Van Emden 1973). *Mylabris pustulata* exclusively feeds on the petals of flowers of plants and never feeds on leaves. The results presented in this paper indicate the nutritive relationship between two different flowers and fecundity of *M. pustulata.*

2. Materials and methods

M. pustulata collected from local gardens in the vicinity of Madurai were reared in the laboratory on flowers of *Ipomoea cornea* and *Ipomoea tuberosa* at 30 ± 1°C and 70 RH. Beetles were fed *ad libitum* every day.

2.1 *Fecundity studies*

A pair of male and female (400–450 mg) insects were selected from the culture and introduced in a plastic container (10 × 6 × 6 cm) and fed *ad libitum* with concerned flower. Six replicates were maintained for each flower. The eggs laid by each female insect were recorded.

2.2 *Biochemical studies*

The protein, carbohydrate and lipid contents in the flowers of food plants were estimated following the methods of Lowry *et al* (1951), Seifter *et al* (1950) and Bragdon (1951) respectively. Water content in the leaves of each food plant was measured by drying the fresh flowers in hot air oven at 60°C to a constant weight.

2.3 *Food consumption studies*

The female beetles were fed *ad libitum* on weighed quantities of their respective food for 15 days and 6 replicates for each food were maintained. Unfed leaves and faeces were collected daily and dried at 60°C to find out the percentage of water content. The amounts of food consumed, defecated, assimilated and converted were estimated directly following IBP formula (Petrusewicz and Macfadyen 1970) represented as $C=P+R+(F+U)$ where C is the total food consumed, P the growth, R the energy spent on metabolism and $F+U$ the energy loss via faeces including nitrogenous excretory products.

3. Results and discussion

3.1 *Fecundity of M. pustulata*

Data presented in table 1 indicate that the maximum number of eggs were laid when *M. pustulata* was reared on *I. cornea* (144 ± 30·80) and minimum on *I. tuberosa* (93·33 ± 38·87).

3.2 *Relation between the composition of flowers and fecundity*

The nutritive value of the flowers was assessed by estimating the protein, lipid, carbohydrate and water in them. The concentration of protein, lipid and water was comparatively greater in *I. cornea* than *I. tuberosa* (table 2).

Table 1. Eggs laid per female of *M. pustulata* reared on flowers of *I. cornea* and *I. tuberosa*.

Food plants	Fecundity
I. cornea	144·00 ± 30·80
I. tuberosa	93·33 ± 38·87

Each value represents the average performance of 6 individuals (mean ± SD) maintained at 30 ± 1°C.

Table 2. Composition of the flowers of *I. cornea* and *I. tuberosa* expressed in percentage.

Food plants	Carbohydrate	Protein	Lipid	Water
I. cornea	5·3 ± 0·16	4·88 ± 0·04	6·0 ± 1·6	89·95 ± 1·20
I. tuberosa	5·8 ± 0·04	4·64 ± 0·09	1·9 ± 0	88·62 ± 0·90

The high fecundity observed on *I. cornea* may be due to its high protein content (4·88%) than *I. tuberosa* (4·64%). High protein and nitrogen content and low sugar in *Luffa cylindrica* were said to be the causative factors for the increased fecundity in the pest *Cyrtopeltis tenuis* (Raman and Sanjayan 1984). In the case of the seed bugs, *Oxycarenus hyalinipennis* and *Spilostethus hospes* maximum egg production was achieved on the host plant seed which contained higher proteins, nitrogen and a narrow C/N ratio (Ananthakrishnan *et al* 1982a, b).

Data were statistically analysed and a high correlation was found between protein content and fecundity ($r = 0{\cdot}9748$) which showed a significant fit at 0·05 level (figure 1). Slansky (1982) found that protein is required not only for adult maintenance but also to supply the energy and nutrients for provisioning the eggs and egg production in insects.

3.3 *Feeding budget of M. pustulata*

Recent studies have indicated that not only the quality but the quantity of food consumed by the insect may also have an effect on fecundity. Data given in table 3 indicate that all the feeding parameters viz food consumption, assimilation, conversion and feeding rate were greater in *M. pustulata* maintained on *I. cornea*. Statistical analysis showed that fecundity of *M. pustulata* depended on the

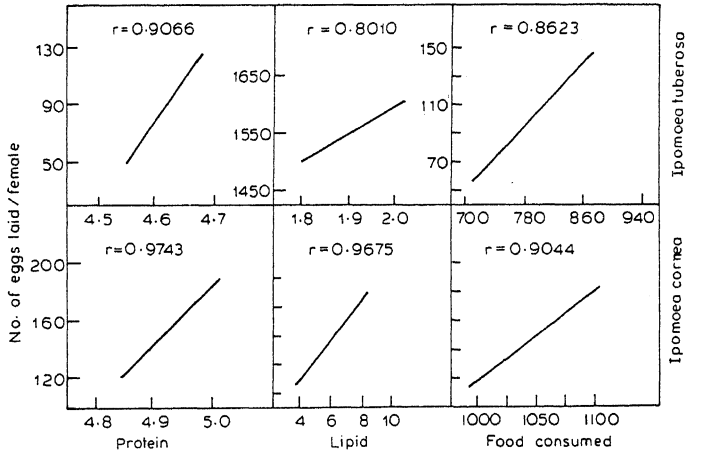

Figure 1. Regression to describe the relationship of protein and lipid content of food plants and food consumption by *M. pustulata* against fecundity.

Table 3. Feeding budget of *M. pustulata* (♀) fed *ad libitum* on flowers of *I. cornea* and *I. tuberosa*.

Food plants	Food consumed	Faeces produced	Food assimilated	Food converted	Food metabolized	Feeding rate (mg/g live wt/day.)
I. cornea	1055 ± 32·58	447 ± 26·67	608 ± 23·53	83 ± 14·66	525 ± 36·23	330 ± 10
I. tuberosa	784 ± 55·93	312 ± 35·07	472 ± 34·82	37 ± 9·40	435 ± 32·27	110 ± 09

Each value represents the average performance of 6 individuals (mean ± SD) (mg/dry wt) maintained for a period of 15 days at 30 ± 1°C.

consumption of *I. cornea* ($r = 0{\cdot}9044$; $P < 0{\cdot}01$). Mukerji and Guppy (1970) also reported that the fecundity increased with the rate of food intake in *Pseudaletia unipuncta.*

This study highlights that the protein content of the food plants and the quantity of food consumed essentially determine the fecundity in phytophagous insects.

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Effect of temperature and host seed species on the fecundity of *Callosobruchus maculatus* (F.)

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Abstract. *Callosobruchus maculatus* was reared on *Vigna unguiculata*, *Phaseolus radiatus* and *Dolichos lab lab* from hatching to death at 20, 25, 30 and 35°C. Food consumption, growth and egg production were estimated in terms of energy. At the tested temperatures, host seed species significantly influenced feeding and energy allocated to egg production. Irrespective of the seed species, food consumption decreased with increasing temperature. Energy density of an egg did not vary with seed species but significantly decreased with increasing temperature. Fecundity (egg/females) of *Callosobruchus maculatus* ranged from 73 on *D. lab lab* at 35°C to 118 on *Vigna unguiculata* at 30°C. Energy allocated to egg production at 20°C was more than at other temperatures. A statistically significant inverse relationship exists between overall feeding rate of *Callosobruchus maculatus* and energy allocated to egg production.

Keywords. Feeding; fecundity; egg production; efficiency; temperature; seed species effect; *Callosobruchus maculatus*.

1. Introduction

Callosobruchus maculatus (Coleoptera: Bruchidae) infests a variety of pulses such as bengal gram, green gram, black gram and pigeon pea and inflicts extensive deterioration of the quantity and quality of the seeds. Considerable information is available on the oviposition behaviour and fecundity of stored grain pests. Srivastava and Bhatia (1959) and Gokhale and Srivastava (1976) have reported ovipositional preference of *C. chinensis* and *C. maculatus* on several leguminous seeds (see also Saxena *et al* 1971; Bhattacharya *et al* 1977). These authors have related number of eggs oviposited to nature (smooth and rough) or area of seed surface available for oviposition. They have totally ignored the nutritional adequacy of the tested seeds as a factor in determining the fecundity of the pests. Leelavathy (1982) and Mark (1982) have stressed the importance of nutritional quality of seeds in determining ovipositional preference and fecundity (see also Fox and Morrow 1981). Presence of oviposition and/or feeding deterrents in the seed is also an important factor in determining fecundity (see Applebaum and Guez 1972; Janzen *et al* 1976, 1977). Klekowski *et al* (1967) and Campbell *et al* (1976) have studied the pattern of transformation of food in *Tribolium castaneum* and *Sitophilus granarius*, respectively. The species of bruchids studied by them do not bore into the seeds but feed from outside throughout their life time (except during the pupal period). *C. maculatus* and *C. analis* bore into the seeds and feed till the completion of larval development. Therefore, the pattern of utilization of food and energy allocation to egg production in *C. maculatus* is likely to be different from *T. castaneum* and *S. granarius*. The

present paper reports effects of seed species and temperature on feeding and energy allocation to egg production in *C. maculatus*.

2. Materials and methods

Freshly emerged *C. maculatus* (5 ♀♀ and 5 ♂♂) were allowed to oviposit on 100 seeds each of *Vigna unguiculata*, *Phaseolus radiatus* and *Dolichos lab lab* at 20, 25, 30 and 35°C and 75% RH. Seeds of similar size were taken in 5 cm dia petridishes and exposed to *C. maculatus* females for oviposition. Subsequent to oviposition, seeds with single egg were separated, weighed individually, numbered and transferred to glass vials. Ten replicates of 200 seeds of each species with one egg on each seed were maintained at the desired temperatures in BOD incubators. Simultaneously, 100 seeds of more or less the same weight but without any eggs were maintained at the tested temperatures as control. To ascertain hatching and moulting to higher instar, a minimum of 5 infested seeds from each replicate were dissected every day and head capsule width of the larva inside was measured. Using the data on head capsule width for the different larval instars reported by Sharifi and Mills (1971), the larval instars were determined. On the day of moulting to the next instar, unfed seed remains, egesta and exuvia were separated and dried. Subtracting the dry weight of the unfed remains at the end of an instar from the dry weight of the seed at the commencement of the instar, food consumption for the instar was determined. Total consumption for the entire larval period was obtained by adding the food consumption for the different instars. Overall feeding rate (KJ g^{-1} live insect d^{-1}) was calculated as follows

$$\text{Feeding rate (Cr)} = \frac{\text{Food consumed (KJ/instar)}}{\text{Midbody weight (g)} \times \text{duration of the instar (day)}}.$$

$$\text{Overall Cr} = \frac{\text{Cr of instar I} \times \text{duration} + \text{Cr of instar II} \times \text{duration} \ldots \text{instar IV}}{\text{Total larval period (day)}}.$$

Somatic growth was estimated as the difference between the dry weight of the freshly hatched larva and that of the terminal larva. Freshly mated females were exposed to the respective seed species on which they were reared for oviposition. Number of eggs oviposited by a female and the egg weight were estimated. Samples of food, test insects and eggs were dried to constant weight at 75°C and energy content of the samples was estimated in a Parr 1421 bomb calorimeter. Egg production efficiency (%) was calculated as follows

$$\text{Egg production efficiency (\%)} = \frac{\text{Energy allocated to egg production (J/insect)}}{\text{Food energy consumed (J/insect)}}$$

3. Results and discussion

Data on food consumption and energy allocated to somatic growth and reproductive growth by *C. maculatus* developing in the different species of seeds tested are provided in table 1. Irrespective of the seed species, food consumption decreased with increasing temperature. However, due to shortening of larval period at higher

Table 1. Feeding and egg production of *C. maculatus* developing in *V. unguiculata*, *P. radiatus* and *D. lab lab*.

Parameters	*V. unguiculata*				*P. radiatus*				*D. lab lab*			
	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C	20°C	25°C	30°C	35°C
Larval duration (day)	23·5	19·5	16·0	12·0	26·0	22·0	17·5	13·5	45·5	36·0	30·0	26·0
Food consumption (J/insect)	444·24	419·34	386·23	366·69	467·50	444·93	421·26	405·56	1207·58	1106·55	1040·22	902·79
Feeding rate (KJ/g/day)	11·53	15·93	22·34	39·25	12·88	16·56	23·91	34·66	16·13	19·39	28·66	38·06
Somatic growth (J/insect)	108·80	97·38	81·66	68·26	88·54	83·25	76·70	63·46	110·06	100·03	85·27	73·42
Mass of egg (μg/egg)	30·1	22·2	19·0	15·0	30·1	22·2	19·0	15·0	30·1	22·2	19·0	15·0
Energy of egg (J/egg)	0·286	0·210	0·181	0·143	0·286	0·210	0·181	0·143	0·286	0·286	0·181	0·143
Number of eggs (No/insect)	102·0	106·6	117·5	87·6	85·0	89·5	95·0	72·5	90·5	95·1	102·0	72·5
Reproductive growth (J/insect)	29·24	22·50	21·24	12·47	24·36	24·36	17·25	10·32	25·80	19·97	18·51	10·31
Egg production efficiency (%)	6·6	5·4	5·5	3·4	5·2	5·5	4·1	2·5	2·1	1·8	1·8	1·1
Life span of adult (day)	13·0	9·0	6·0	5·0	12·0	8·0	6·0	5·0	12·0	8·0	6·0	5·0

Each value represents the average of 10 replicates.

temperatures, feeding rate increased with temperature. For instance, *C. maculatus* developing in *V. unguiculata* consumed 444, 419, 386 and 367 J/insect at the rate of 11·53, 15·93, 22·34 and 39·25 KJ g^{-1} live weight d^{-1} at 20, 25, 30 and 35°C respectively (table 1). Of the 3 species tested, *C. maculatus* developing in *D. lab lab* ingested the maximum food energy at all the temperatures. For instance, at 30°C consumption in *D. lab lab* was 1040 J/insect compared with 386 J/insect for that developing in *V. unguiculata*. Prolongation of larval development in *D. lab lab* is responsible for more than two fold increase in food consumption by *C. maculatus* developing in *D. lab lab* over that developing in the other two tested seed species. The pattern of energy allocation to somatic growth at the tested temperatures was similar to that observed for food consumption. *C. maculatus* realised maximum somatic growth in *D. lab lab*. It ranged from 73·42 J at 35°C to 110·06 J at 20°C (table 1). *P. radiatus* supported the lowest growth at all the tested temperatures. Weight and energy content of a single egg oviposited by *C. maculatus* did not vary with the host seed species but decreased with increasing temperature. The mass of an egg averaged to 30·1, 22·2, 19·0 and 15·0 μg at 20, 25, 30 and 35°C respectively; corresponding values for the energy content were 0·286, 0.210, 0.181 and 0·143 J/egg (table 1). The number of eggs oviposited by *C. maculatus* significantly varied with seed species and temperature. Maximum number of eggs were oviposited by *C. maculatus* reared on *V. unguiculata* at 30°C (table 1).

Of the 3 species of seeds tested, fecundity of *C. maculatus* developing in *P. radiatus* was low (table 1). Consequent to the difference in fecundity of *C. maculatus* in the different seed species, energy allocated to egg production and hence egg production efficiency varied. For instance, at 20°C *C. maculatus* developing in *V. unguiculata* allocated 29·24 J for egg production compared with 25·8 J and 24·36 J by that developing in *D. lab lab* and *P. radiatus*. Egg production efficiency expressed as percentage of ingested food energy allocated to egg production decreased with increasing temperature. The decrease was from 6·6% to 20°C to 3·4% at 35°C for the insect developing in *V. unguiculata* (table 1). Egg production efficiency of the insect in *D. lab lab* was less than that in the other seed species. For instance, the efficiency at 20°C was 6·6% in *V. unguiculata* compared with 5·2% and 2·1% in *P. radiatus* and *D. lab lab* (table 1).

The results presented above clearly indicate that *C. maculatus* maximises egg production in *V. unguiculata* at 30°C. On the basis of egg production efficiency also, *V. unguiculata* and *P. radiatus* appear to be more suitable for feeding and energy allocation to egg production. Although food consumption in *D. lab lab* at the tested temperatures is twice more than that on the other seed species, prolonged larval development and the consequent increase in the expenditure of energy on maintenance metabolism resulted in the restriction of energy available for egg production. Chandrakantha (1985) found that *V. unguiculata* seeds contain more nitrogen, lipids, energy and water than *P. radiatus* and *D. lab lab* (table 2) and that *C. maculatus* utilizes *V. unguiculata* more efficiently than *P. radiatus* and *D. lab lab*. Accumulation of sufficient nitrogen during the feeding larval period is essential to maximise egg production during the non-feeding adult period. Gross nitrogen accumulation efficiency of *C. maculatus* developing in *V. unguiculata* was 71·6% compared with 68·8% and 62·8% for that developing in *P. radiatus* and *D. lab lab* (Chandrakantha 1985). Therefore to maximise egg production *C. maculatus* prefers nitrogen rich *V. unguiculata*, which affords faster development. The presence of

Table 2. Chemical constituents, energy and water content of the host seeds provided to *C. maculatus*.

Seed species	Nitrogen	Lipid	Carbohydrate	Ash	Energy	Water
V. unguiculata	2·7 ± 0·01	1·3 ± 0·04	55·7 ± 0·07	2·9 ± 0·16	19·7 ± 0·16	27·3 ± 0·97
P. radiatus	2·5 ± 0·01	0·9 ± 0·03	56·6 ± 0·04	4·3 ± 0·23	18·9 ± 0·12	25·6 ± 0·94
D. lab lab	1·9 ± 0·02	0·7 ± 0·04	59·3 ± 0·09	4·4 ± 0·20	17·2 ± 0·11	23·2 ± 0·86

Values of chemical constituents and water are expressed in percentage dry weight and energy content in terms of J mg^{-1} dry matter.

saponins and protease inhibitors in *D. lab lab* (see Gokhale 1976; Applebaum and Guez 1972) is likely to interfere with digestion and assimilation of nutrients rendering *D. lab lab* less suitable for food consumption and utilization.

The finding in the present study that *C. maculatus* oviposited more eggs at 30°C in the tested species of seeds conforms with the conclusion of Mookherjee and Chawla (1964) and Giga and Smith (1983). However the obvious increase in the metabolic cost at 30°C resulted in decrease in egg production efficiency and allocation of less energy for egg production than that at 20°C and 25°C. The adult life span of *C. maculatus* at the tested temperatures ranged from 8–13 days. Granivorous insects like *S. granarius* (Campbell *et al* 1976), *Cryptolestes ferrugenius* (Campbell and Sinha 1978) and *Oryzaephilus surinamensis* pass through a prolonged adult life span of over 30 days. These insects feed throughout their life time as against *C. maculatus* which feeds only during the larval period. Whereas *C. maculatus* resorts to shorten the adult duration and maximise egg production, the other granivores shorten the larval duration and allocate most part of the food energy consumed during the adult period for egg production.

Figure 1 shows the inverse relationship between feeding rate and energy allocated

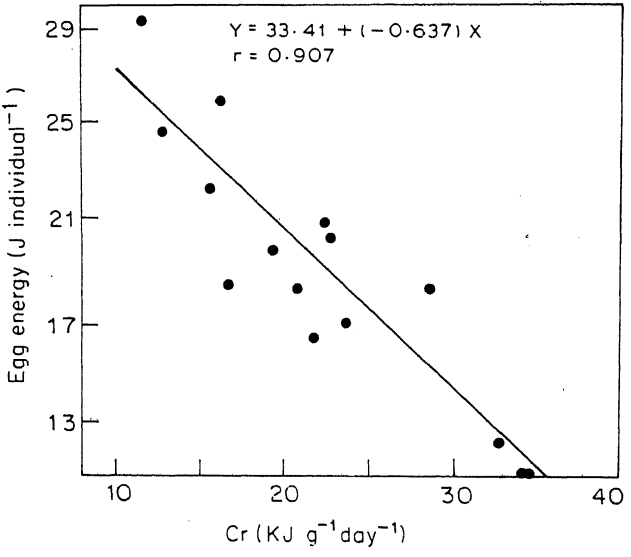

Figure 1. Energy allocated to egg production of *C. maculatus* as a function of overall feeding rate.

Table 3. Observed and predicted values of energy allocated to egg production by *C. maculatus*.

Host seed species	Temp. (°C)	Observed egg energy (J)	Predicted from TL	X^2	Predicted from pupa	X^2	Predicted from imago	X^2
V. unguiculata	20	29·24	25·93	0·374	27·45	0·109	29·89	0·014
	25	22·50	22·97	0·009	23·67	0·060	22·21	0·003
	30	21·24	18·93	0·251	20·17	0·053	20·32	0·039
	35	12·47	15·47	0·721	15·78	0·879	17·17	1·810
P. radiatus	20	24·36	20·79	0·523	21·12	0·043	22·16	0·198
	25	18·71	19·34	0·021	19·30	0·018	18·43	0·004
	30	17·25	17·65	0·009	17·19	0·0002	16·85	0·009
	35	10·32	14·24	1·488	12·87	0·630	13·54	1·004
D. lab lab	20	25·80	26·22	0·006	27·39	0·097	25·77	0·0003
	25	19·97	23·47	0·613	23·02	0·465	22·44	0·305
	30	18·51	19·85	0·097	17·95	0·016	17·20	0·092
	35	10·31	16·77	4·047	12·99	0·696	11·34	0·102

to egg production in *C. maculatus*. The statistically significant correlation between the two variables may be made use of to predict the energy allocated to egg production from feeding rate. The observed and predicted values of energy allocated to egg production by *C. maculatus* developing in the different seed species are provided in table 3. The observed and predicted data do not differ significantly and hence the proposal of prediction of energy allocation to egg production from feeding rate stands valid.

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Nutritional influence on the growth and reproduction in two species of acridids (Orthoptera: Insecta)

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Abstract. Comparative analysis of the effect of different natural host plants on the nymphal duration, quantitative food utilization and fecundity of two species of acridids, *Aiolopus thalassinus* (Fabricius) and *Gesonula punctifrons* Stal revealed significant differences that were attributed to the varied nutritive value of the host plants. *Aiolopus thalassinus* appeared to show greater preference for *Panicum maximum* and *Cyperus rotundus* as against *Cynadon dactylon* and *Coix lachryma*. Similarly *Gesonula punctifrons* showed greater food intake and fecundity on *Eichhornia crassipes* and *Colocasia* sp than *Musa paradisiaca* and *Ipomoea* sp. Various growth parameters such as consumption index, approximate digestibility, growth rate, efficiency of conversion of ingested food and digested food were also analysed for the study of the nutritional impact in addition to the biochemical profile of the host plants.

Keywords. *Aiolopus thalassinus*; *Gesonula punctifrons*; nymphal duration; quantitative food utilization; fecundity; consumption index; growth rate.

1. Introduction

Earlier studies on insect nutrition were mainly focussed on the qualitative food requirements of insects, the basic criteria used in evaluating a diet being based on the time taken for the first instar nymph to reach the adult, gain in body weight and sex ratio. The data present strictly the gross comparative value of food plants and do not make distinction between the nature of food and the basic causes for the failure of development of insects on certain plant species. For a better understanding of the nature of food material, it is obligatory to collect information on the rate of feeding and its effect on growth and development, the amount of food digested, and the quantity of food converted into body mass. Ananthakrishnan *et al* (1986a,b) used such parameters for analysing the host preference of the cotton grasshopper *Cyrtacanthacris ranacea* and also stressed the importance of sensillar density and diversity in the feeding range. The role of physicochemical factors in host selection of grasshoppers have been well recognised (Mulkern 1967; Bernays and Chapman 1977; Sanjayan and Ananthakrishnan 1987). The nutritional influence of different host plants discussed here is based on two criteria: (i) Long term physiological components involving the effect of different hosts on the duration of post embryonic development and fecundity of the acridids and (ii) short term behavioural component in terms of the effect of different host plants on the quantitative food intake and subsequent analysis of various growth parameters. Two species of acridids were chosen for this study namely *Aiolopus thalassinus* (Fabricius) and *Gesonula punctifrons* Stal, both being restricted oligophagous grasshoppers feeding mainly on monocots and completely avoiding the dicots.

2. Materials and methods

A. thalassinus and *G. punctifrons* were field collected from their respective host plants and reared in cages measuring 25 × 25 × 30 cm. Newly hatched first instar nymphs were segregated in groups and each group was fed with any one of the host plants. Observations were made on the duration spent by each instar on the different host plants. The total nymphal duration on each test plant was taken as an index for comparison of the rate of development.

2.1 *Quantitative food utilization*

The gravimetric method was adopted for assessing the quantity of food utilized. Experimental insects were separated from the stock culture, confined individually and provided with water to clear off their guts for a period of 10 h. After measuring the initial weight, the grasshoppers were introduced one per cage and allowed to feed on weighed quantity of the host plant leaves for a period of 24 h. At the end of the experiment the left over food, excreta and the insects were weighed. The difference in the weight of the insect gives the weight gained during the period of study. Comparison of the various food utilization was carried out by studying the growth parameters of Waldbauer (1968).

2.2 *Biochemical estimations*

The host leaves were subjected to biochemical estimations for their total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956), nitrogen (Vogel 1963) and phenol (Hori 1974). For analysis of the qualitative profiles of amino acids in the different host leaves, Hewlett Packard High Performance Liquid Chromatography (HPLC) was used. O-phthalaldehyde derivatives of the amino acids in the alcoholic extract was chromatographed (Lindroth and Mopper 1979) using Hypersil ODS 5 μm column. 0·1 M phosphate buffer pH 7·7 and methanol were used as mobile phase and fluorescent derivatives were detected at 340 nm. The amino acids were identified based on standard chromatograms of individual amino acids and the area per cent individual amino acid peaks were calculated.

3. Observations

3.1 *Duration of post embryonic development*

Data on the duration of nymphal development and fecundity of the two acridid species is provided in table 1. Maximum fecundity and fastest rate of development of *A. thalassinus* were observed on *Cyperus rotundus* followed by *Panicum maximum*, *Cynadon dactylon* and *Coix lachryma*. For *G. punctifrons*, *Eichhornia crassipes* appeared to facilitate the highest fecundity as well as the shortest nymphal duration. No eggs were laid for acridids fed on *Musa paradisiaca*. Based on the data on the fecundity and duration of post embryonic development, the host plants could be arranged in the order of their preference as follows: (i) *A. thalassinus*: *C. rotundus* >

Table 1. Impact of host plants on duration of post embryonic development and fecundity.

	Total nymphal duration	Fecundity
A. thalassinus		
P. maximum	43 ± 0·53	61·2 ± 3·2
C. rotundus	40 ± 0·45	72·3 ± 2·5
C. dactylon	49 ± 0·63	33·5 ± 6·8
C. lachryma	51 ± 0·98	14·1 ± 4·4
G. punctifrons		
E. crassipes	40·5 ± 3·32	39·0 ± 1·09
Colocasia sp	42·8 ± 2·68	26·4 ± 0·89
M. paradisiaca	41·0 ± 1·41	—

Values represent mean ± SD of 6 replications.

P. maximum > *C. dactylon* > *C. lachryma*; (ii) *G. punctifrons*: *E. crassipes* > *Colocasia* sp > *M. paradisiaca*.

3.2 *Quantitative food intake*

Tables 2 and 3 provide data on the consumption and utilization of different host leaves by the various instars as well as adults of *A. thalassinus* and *G. punctifrons* respectively. Analysis of the amount of food ingested showed variations not only with the host plants but also among the developmental stages of the acridids. The data of the fifth instar nymphs have been taken for comparison of the host plants with regard to their utilization. The maximum weight of food ingested by *A. thalassinus* was from *C. rotundus* (248·10 mg/g) in comparison to the other hosts. *G. punctifrons* ingested more of *E. crassipes* (188·0 mg/g) followed by *M. paradisiaca* (172·0 mg/g) and *Colocasia* sp (121·5 mg/g). The weight gained was also the highest on the most preferred host. Various growth parameters were computed for an understanding of the nutritive value of the host plants. In general the acridids showed a decrease in the consumption index with the advancement of the nymphal stages. On all the host plants the consumption index (CI) of the first and second instars of *A. thalassinus* was comparatively higher than the other nymphal stages. The growth rate was also significantly higher in the early nymphal instar. A comparison of the data on the approximate digestibility (AD) indicate that the digestibility of all the plants tested to be fairly high and this could explain the acridid completing a number of generations even on a host plant of lesser nutritive value. Comparison of the efficiency of conversion of ingested food and digested food values of the fifth instars fed with various host plants indicates the efficiency of ingested and digested food to be high on the lesser preferred host.

3.3 *Biochemical correlates*

Table 4 provides data on the chemical analysis of the various host plants of the two acridids in terms of their total protein, carbohydrates, phenols, nitrogen and water. A comparison of the hosts of *A. thalassinus* indicate the preferred host plant, *C. rotundus*, to have lesser amount of proteins, carbohydrates, phenols and nitrogen.

Table 2. Quantitative food utilization of *A. thalassinus*†.

Host plants	Instars	Wt of food ingested*	Wt of excreta*	Wt gain by insect*	CI	AD	GR	ECI	ECD
P. maximum	I	69·76	26·17	1·93	15·7338	62·49	0·4386	2·766	4·428
	II	77·40	34·70	2·93	10·4594	55·17	0·3959	3·785	6·861
	III	73·13	3·60	6·73	4·5700	95·39	0·3938	8·613	9·029
	IV	65·13	21·96	7·83	1·3500	66·28	0·1629	12·022	18·138
	V	117·60	38·21	19·66	1·0257	67·51	0·1715	16·72	24·760
	Adult ♂	153·43	23·70	20·76	1·1400	84·55	0·1549	13·53	16·002
	Adult ♀	212·32	78·33	32·65	0·7162	63·11	1·1016	15·38	24·370
C. lachryma	I	57·00	10·06	1·60	13·1639	81·40	0·3695	2·807	3·448
	II	42·60	9·30	4·10	7·0413	78·16	0·6776	9·624	12·312
	III	124·20	23·73	10·40	8·5419	80·89	0·7162	8·373	10·351
	IV	82·80	30·03	5·03	2·6058	63·73	0·1583	6·070	9·530
	V	58·36	34·16	29·43	0·5466	41·47	0·2756	50·430	21·610
	Adult ♂	89·25	38·15	32·34	0·4354	57·25	0·1578	36·240	74·660
	Adult ♀	122·34	42·23	38·69	0·5319	65·48	0·1682	31·620	48·300
C. rotundus	I	85·70	13·62	2·23	16·3260	84·10	0·4248	2·602	3·093
	II	108·30	24·82	3·42	17·8125	77·08	0·5625	3·157	4·096
	III	35·96	6·70	11·33	1·7182	81·37	0·5414	31·51	38·720
	IV	123·60	60·86	13·53	1·5159	50·76	0·1660	10·95	21·570
	V	248·10	78·51	20·22	2·6357	68·32	0·2148	8·15	11·920
	Adult ♂	420·31	62·45	30·31	1·3254	85·14	0·1932	7·21	8·470
	Adult ♀	489·05	72·31	35·65	1·0586	85·21	0·1435	7·29	8·550
C. dactylon	I	54·25	11·97	2·03	19·6701	88·96	0·3680	1·871	2·102
	II	68·50	22·35	3·52	6·1823	67·37	0·3176	5·138	7·627
	III	72·30	14·38	5·21	4·2280	80·11	0·2547	7·210	8·995
	IV	27·93	11·11	10·66	0·4624	60·22	0·1768	38·17	63·370
	V	151·80	117·93	13·66	1·1632	22·31	0·1047	8·99	40·330
	Adult ♂	182·52	82·25	25·33	0·7935	54·94	0·1101	13·88	25·260
	Adult ♀	203·31	91·35	38·66	0·6558	55·07	0·1247	19·02	34·530

†Mean of 5 replicates, *mg/day/insect.

Table 3. Quantitative food utilisation of *Gesonula punctifrons*†.

Host	Instar	Wt of food ingested*	Wt of excreta*	Wt gain by insect*	CI	AD	GR	ECI	ECD
E. crassipes	I	50·05	18·00	10·0	0·290	64·00	0·117	5·05	31·25
	II	207·62	105·46	6·0	3·334	49·28	0·066	34·32	5·87
	III	128·65	118·90	8·0	2·146	7·82	0·065	15·01	80·00
	IV	229·45	99·00	7·0	1·569	56·77	0·379	29·86	5·38
	V	188·00	90·05	12·5	0·564	83·06	0·024	15·71	12·24
	Male adult	202·05	198·05	16·5	0·176	1·98	0·029	16·88	37·50
	Female adult	392·05	328·06	18·7	0·997	16·33	0·029	21·78	15·63
M. paradisiaca	I	81·50	57·00	2·0	0·166	29·62	0·003	0·01	2·47
	II	217·51	113·72	6·5	3·209	61·76	0·065	33·46	6·27
	III	154·32	102·12	4·5	0·243	27·28	0·004	34·30	8·63
	IV	282·19	199·34	3·5	0·236	50·53	0·010	80·63	0·78
	V	172·08	126·50	4·5	0·967	26·75	0·008	38·29	11·75
	Male adult	366·05	142·05	5·7	1·246	73·05	0·008	66·56	0·97
	Female adult	512·01	156·00	6·0	1·060	57·38	0·011	78·78	1·09
Colocasia sp	I	96·02	30·05	8·0	15·002	71·87	0·096	0·07	8·34
	II	99·05	33·06	7·5	1·420	43·36	0·074	0·06	7·07
	III	111·05	55·12	6·8	1·524	51·75	0·057	0·04	5·40
	IV	116·36	74·56	13·4	0·068	72·05	0·064	0·07	31·10
	V	121·50	83·05	11·0	0·027	18·00	0·030	25·38	55·11
	Male adult	393·51	176·92	15·5	0·270	21·00	0·036	35·56	43·35
	Female adult	530·63	183·38	15·6	0·150	13·24	0·042	42·78	52·80

†Mean of 5 replicates. *mg/day/insect.

However, the water-nitrogen index computed was significantly high. A reverse trend was observed for these parameters on the preferred host of *G. punctifrons* namely *E. crassipes*. *E. crassipes* was comparatively rich in proteins, carbohydrates and nitrogen but had a very low water-nitrogen index. Figures 1 and 2 provide the HPLC analysis for amino acids of the various host plants. It is interesting to note that histidine and lysine were present in all the host plants of *A. thalassinus*. Aspergine, arginine, alanine, tyrosine, cysteine, tryptophan, methonine and leucine

Table 4. Biochemical analysis of host plants of the two acridids.

Host plants	Proteins (mg/g)	Carbohydrates (mg/g)	Phenols (mg/g)	Nitrogen (%)	Water (%)	Water/ nitrogen
C. rotundus	42·0	193	73·5	6·72	71·74	10·67
C. dactylon	55·5	583	102·0	8·88	61·97	6·97
P. maximum	159·0	586	160·5	9·28	77·29	8·32
E. crassipes	130·0	66	300·0	7·80	82·25	10·54
M. paradisiaca	10·0	36	460·0	1·60	68·92	43·08
Colocasia sp	8·0	30	250·0	1·28	77·35	60·43

Mean of 3 replicates.

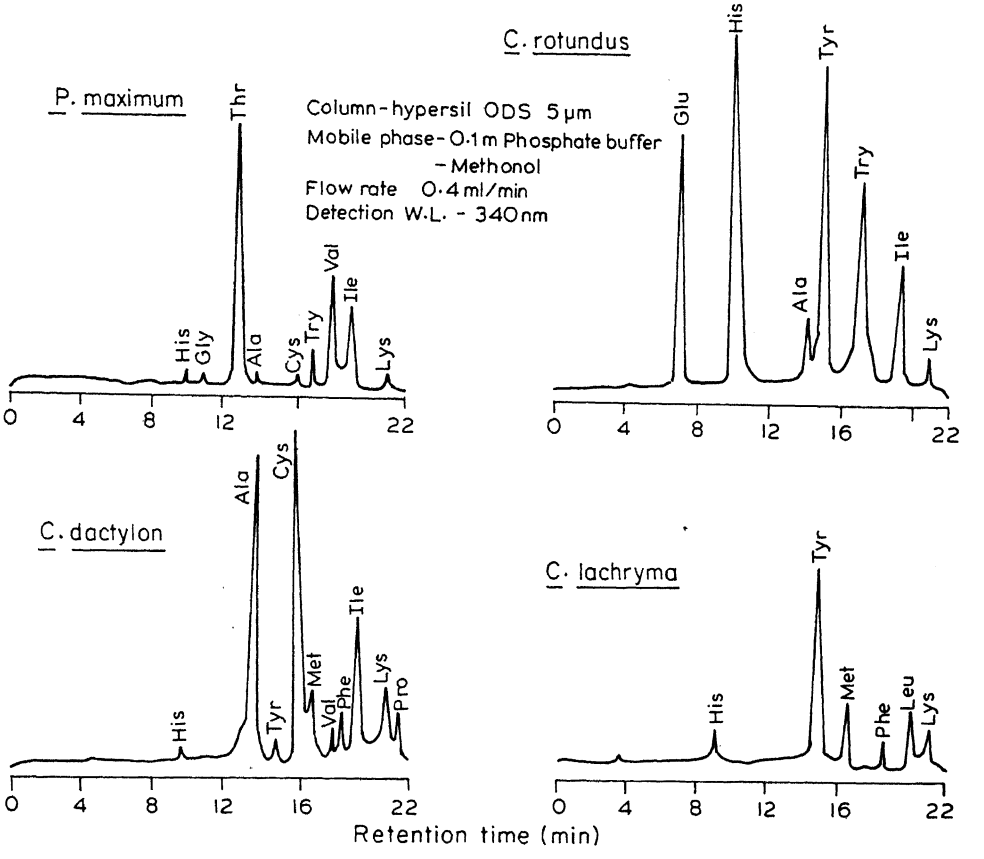

Figure 1. HPLC analysis of amino acids.

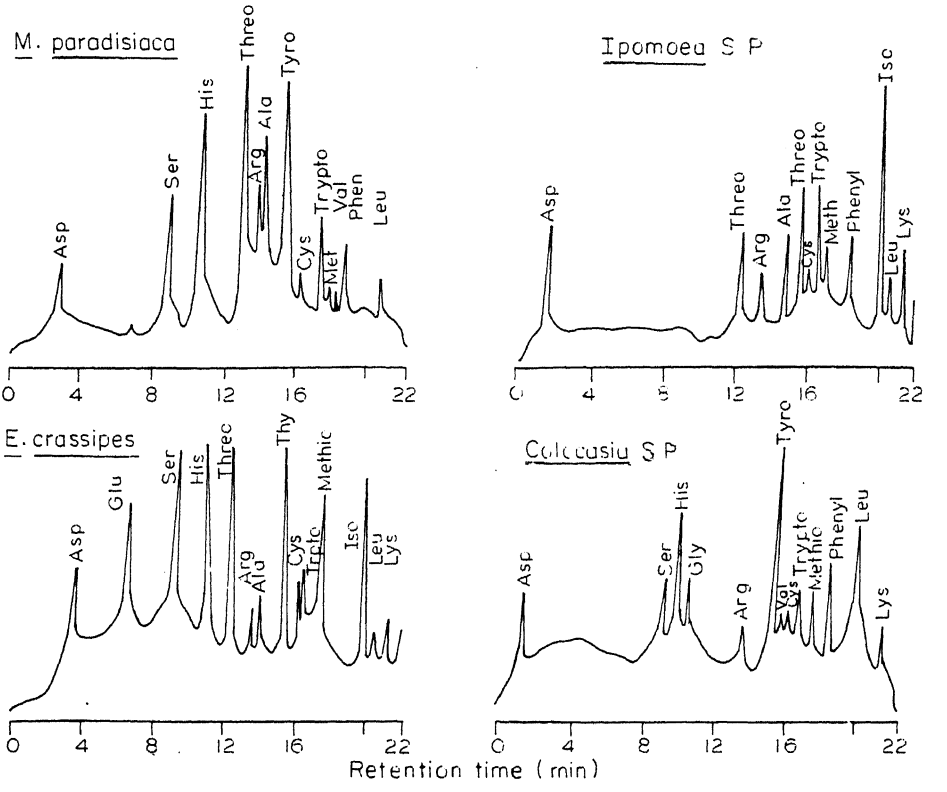

Figure 2. Same as in figure **1**.

were present in all the hosts of *G. punctifrons*. The presence of glutamine at a high percentage only on the preferred host, *C. rotundus*, may play an important role in the greater performance of the acridids reared on *C. rotundus*.

4. Discussion

The interactions between insects and plants is a complex process involving analysis of the responses of insects to plants as well as those plant characteristics which evoke these responses. Although it is known that plants contain all the essential nutrients required by insects, in nature an insect does not feed on all the plants. At the same time a plant in a particular habitat is not attacked by all the insect species in that habitat. This shows that there is some degree of association between the insects and plants such that the insect feeds on certain species of plants or certain groups of plants. The relationship between the acridid and its host plant is studied here based on such responses of the acridids as the quantitative intake of different host leaves, growth and fecundity, while the characteristics of the plants which evoke the responses of acridids relates to the biochemical composition of the plants in addition to the physical nature of the leaves.

After an insect has located its host plant and has begun to feed, the two variables that interact are the nutritional requirements of the insects in terms of the qualitative

and quantitative aspects and the digestibility and nutritive quality of the host plant (House 1969). The digestibility and nutritive quality of plants vary with insects as also the nutritive requirements of insects. However, all insects are found to have quite a similar qualitative requirements, and it is very likely that all the plants contain 25 essential nutrients required by insects; it appears that qualitative factors may not play a decisive role in the relationship between insects and plants. More likely, the quantitative factors are most important. The quantity of food utilized by the two acridids studied here varied with the host plants. While the food ingested by *A. thalassinus* was greatest on *C. rotundus*, followed by *P. maximum*, *C. dactylon* and *C. lachryma*, the food ingested by *G. punctifrons* was highest on *E. crassipes* in comparison to *M. paradisiaca* and *Colocasia* sp. The weight gained by the insect as a result of feeding on a particular host plant would give a measure of the nutritive value of the host plant. It gives a direct correlation of how much the ingested food have been converted into the body mass. The weight gained by the two acridids was also the highest on the most preferred host indicating that for *A. thalassinus* and *G. punctifrons*, *C. rotundus* and *E. crassipes* respectively had a high nutritive value. Various growth parameters were computed for an understanding of the nutritive value of the host plant. In general the acridids showed a decrease in the consumption index (CI) with the advancement of the nymphal stages. A general trend in the decrease in CI in succeeding instars was also recorded for *S. gregaria* and *L. migratoria* fed with cabbage (Mehrotra *et al* 1972). On all the host plants the CI of the first and second instars of *A. thalassinus* was high compared to the other developmental stages. Comparison of the CI of the fifth instars on various host plants showed the value to be highest on *C. rotundus* and the least on *C. lachryma*. On good host plants the CI was very high while it was too low on poor host plants. Therefore it can be stated that the CI increased with increase in nutrient levels. The early nymphal stages showed the highest growth rate on all the host plants. The approximate digestibility computed indicated all the host plants provided were highly digested by the acridids. The efficiency of conversion of the ingested and digested food was significantly high in the less preferred host thereby enabling the insect to complete its development and reproduce when fed on the lesser preferred hosts.

Faster post-embryonic development of *A. thalassinus* and *G. punctifrons* when fed on *C. rotundus* and *E. crassipes* respectively holds good with the observations of Ananthakrishnan *et al* (1986a) where they mentioned nymphs of *Cyrtacanthacris ranacea* had the shortest duration in the most preferred host. This effect of food on the insect may be either due to the differences in the amount of food ingested or due to the nutritive value of the host plant (Johansson 1964).

To further understand the nutritive differences that exists among the host plants, biochemical estimation of the plants for the total proteins, carbohydrates, nitrogen, phenols and aminoacids yielded significant results: *C. rotundus*, the most preferred host of *A. thalassinus* had a lower quantity of proteins, carbohydrates and phenols in their leaves in comparison to the other hosts. The percentage of nitrogen was also less in *C. rotundus* but the water-nitrogen index computed was the highest. Of particular significance was the HPLC analysis of host plants for the individual amino acid composition. Of significance was also the detection of glutamine only in *C. rotundus* which probably would explain its greater preference by *A. thalassinus*. *Schistocerca gregaria* and *Locusta migratoria* respond differently to protein and

amino acid additions to their diet. Casein provides an adequate source of amino acids for *S. gregaria* but *L. migratoria* fails to develop beyond the third instar (Dadd 1960). Good growth occurs in a diet when protein constitutes 20–40% of dry weight (Dadd 1960). Carbohydrates are essential as a source of energy to acridids and cannot be replaced efficiently by addition of protein or lipid (Dadd 1960). Acridids have no absolute requirement for lipids except for a sterol and some amount of fatty acid namely linoleic or linolenic acid.

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Correlation of nutritional changes with the reproductive potential of *Aphis gossypii* Glover on egg plant

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Abstract. Brinjal (*Solanum melongena* Linn.) leaves of varied maturity show a remarkable variation in respect to the retention of nutrient contents therein. The contents also vary along with the increase of plant age. *Aphis gossypii* Glover, an important pest of the crop, shows high reproductive potential on old and young leaves when the plants are young (2–4 months old) and mature (4–6 months old) respectively. Amongst the nutrient content of the leaves, nitrogen level shows a significant correlation with the reproductive potential of the aphid species leading to its population outbreak and niche selection.

Keywords. Brinjal leaves; *Aphis gossypii*; nutrient contents; reproductive potential; niche selection.

1. Introduction

Nutritional composition of the host plant appears to be a dominating factor in the dynamics of insect-plant-interactions ensuring feeding and oviposition of the insects. Host tissues are related with the nutritional (Ananthakrishnan *et al* 1982; Raman and Sanjayan 1984) and non nutritional (Reese 1929; Rosenthal and Janzen 1979) attributes which reflect the said behavioural responses. Of the phytophagous insects, aphids are too much sensitive to the nutrient values of the food plants and their quantitative availability may also act rapidly on their developmental switch mechanism (Lees 1966; White 1972). Thus, host-plant condition appears as a complement in nutritional ecology of aphids. High correlation between the reproductive activity of aphid species and nitrogen content of the host leaves has been established in *Brevicoryne brassicae* and *Myzus persicae* (van Emden and Bashford 1969) and *Drepanosiphum platanoides* (Dixon 1966).

The present study is an attempt to find out the appreciable correlation, if any, between the nutrient quality of host leaves and reproductive activity of egg plant-aphid, *Aphis gossypii* Glover during kharif season.

2. Materials and methods

In a plot (5 m × 4 m) in Hooghly district (West Bengal) 20 plants of egg plant (brinjal) (Pusa purple cluster variety) were planted during July 1985–86 (kharif season) when the plants were one month old. Observations on population incidence of aphid, *A. gossypii* were made on 3 randomly selected leaves (old, young (mature) and tender) per plant at weekly interval. The plants were uprooted at the end of December. Population distribution of the aphid (%) and its reproductive potential were analysed.

$$r = \frac{\log_e Nt_2 - \log Nt_1}{(t_2 - t_1)}$$

Estimation on reproductive potential (index *r*) of the aphid species was adopted after the methods of Chapman (1928), Birsh (1948) and Odum (1971).

To analyse the major nutrient contents (carbohydrate, total nitrogen, fat, sterols and inorganic salts) dried and powdered leaves (200 g) of egg plant was thoroughly extracted with chloroform in Soxhlet apparatus. The crude extract (2 g) after removal of the solvent was chromatographed on a silica gel column using solvents of increasing polarity. Residues from eluents of different solvents were analysed (Ananthakrishnan 1986) and their approximate quantities determined. The said experiments were repeated in favour of old, young and tender leaves taking from the plants of 2, 4 and 6 months old during the experimental period.

3. Results

3.1 *Aphid incidence*

There was a gradual decrease in the population incidence of aphid (figure 1) in the old leaves while in young and tender leaves it was found to increase gradually towards harvest. Again the greater population volume of the aphid appeared in old, young and tender leaves during premature (2 months old), mature (4 months old) and post mature (6 months old) periods of the plant respectively.

3.2 *Reproductive potential*

Data on the reproductive potential or natality rate (figure 2) reveals that the aphid showed variations in the reproductive rate at different age segments of the brinjal crop. Same was also seen in old, young and tender leaves at different ages of the crop. Again, the aphid appeared with high reproductive potential in old and young leaves when the crop was 2–4 and 4–6 months old respectively. Furthermore, the high reproductive potential of the aphid was found to be associated with the incidence

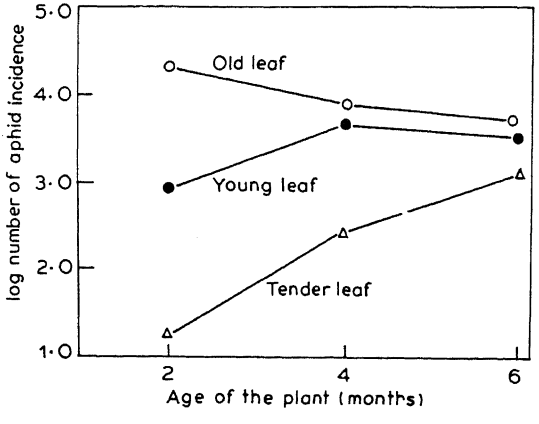

Figure 1. Population incidence of *A. gossypii* on old, young and tender leaves of brinjal at different age of the plant in kharif season during 1985–86.

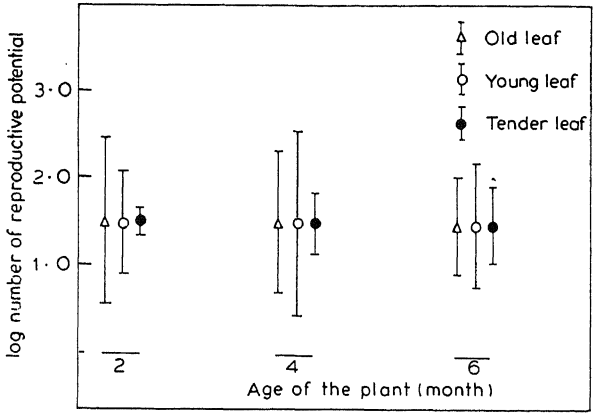

Figure 2. Reproductive potential of *A. gossypii* on old, young and tender leaves of brinjal plant during kharif season (1985–86).

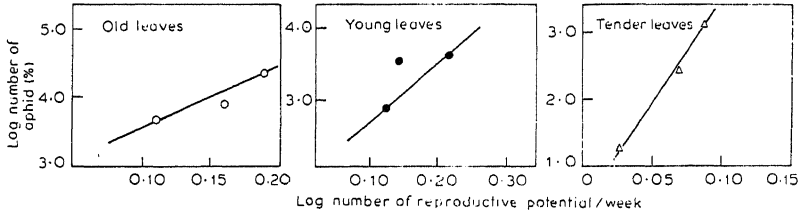

Figure 3. The relationship between the population incidence and reproductive potential of *A. gossypii* on old, young and tender leaves of brinjal plant in kharif season (1985–86).

on the mentioned leaf niches (figure 3). The slopes of the regression lines relating to the reproductive activity and population volume (%) of the aphid showed a significant correlation ($P > 0{\cdot}05$), when the regression equations were recalculated using the common coefficients, the relationship between the aphid population (x) and reproductive potentials (y) appeared in the following forms

(i) In old leaves : $-4{\cdot}59 + 1{\cdot}19x$
(ii) In young leaves : $-0{\cdot}12 + 0{\cdot}07x$
(iii) In tender leaves : $-0{\cdot}01 + 0{\cdot}03x$.

3.3 *Nutrient status of the leaves*

There was a marked difference in the major chemical components of the leaves (old, young and tender) at a particular age of the plant (figure 4). Of these nutrients carbohydrate, nitrogen content and fat appeared in greater proportion in young leaves while the sterols and inorganic salts abundantly appeared in old leaves. Again, most of these nutrients were in maximum in old leaves during the young age (2–4 months) of the plant while the same was seen in young and tender leaves at the old age (4–6 months) of the plant. Furthermore, studies on the relationships of the said leaf nutrients with the reproductive potentials of *A. gossypii* (table 1) reveal that

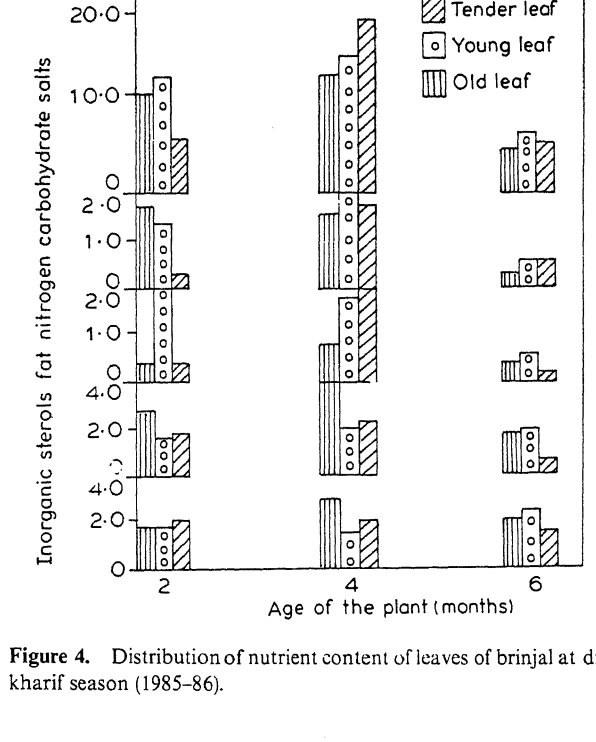

Figure 4. Distribution of nutrient content of leaves of brinjal at different age of the plan kharif season (1985–86).

Table 1. Correlation coefficients between the reproductive potentials of *A. gossypii* a nutrient contents of the leaves.

Different leaves	Nutrients of the leaves				
	Carbohydrate	Nitrogen	Fat	Sterol	Inorganic sal
Old	−0·280	0·792*	−0·333	−0·765	−0·553
Young	−0·079	0·889*	−0·812	−0·111	−0·917
Tender	0·435	0·910*	−0·235	−0·718	−0·089

*Significance at 5% level.

the nitrogen content of the leaves appear to show significant correlation and that w true for all the 3 types of leaves.

4. Discussion

It is evident from above that population density and reproductive potential of apl are extremely varied, as varied as the nutrient content of the leaves along with t increase of crop age. Thus, variation in the nutrient content of the leaf niches (c young and tender) of egg plant indicate an impending condition of host plant parts therein. Positive correlation between the nitrogen content of the leaves and reproductive activity of the aphid species appear to show a preferential response aphid to nitrogen content of the leaves on which the aphid feeds. Again, the variati in the natality of the said aphid species in accordance with the natality of the h

plant deserves a considerable importance. However, it is apparent that the survival and reproduction of insects are bound to be influenced by the quantity and quality of nitrogen present in the tissues which are subjected to variation with the host plant age (Raman and Ananthakrishnan 1986). Again, the reproductive activity of different aphid species has been found to vary at different age of the host due to the changes of amino acid contents of the tissue concerned (van Emden and Bashford 1969; Daiber 1970; Harrewijn 1970; El Ibrashy *et al* 1972). Therefore, it appears to be possible for *A. gossypii* to present a high reproductive activity by changing the niches from old to young to have greater amount of nitrogen content during the increase of crop age. This study though does not conclusively demonstrate the key factor responsible for reproductive response of *A. gossypii*, but the present observation is in well conformity with the findings of Kennedy and Booth (1951), Dixon (1971), Sogawa (1971), Cheng and Pathak (1972), Mc Neill (1973) and Mc Neill and Southwood (1978) about the nutrient discrimination of insects in host selection to exert greater reproductive performance.

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Influence of nutrition on the reproductive biology of sugarcane pests and their natural enemies

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Abstract. The survival and fecundity of the scale insect, *Melanaspis glomerata* (Green) varies according to the sugarcane varieties on which they feed. When the sugarcane setts infested with scale insect crawlers were maintained on different nutritional bases, high survival and fecundity were observed in distilled water as the medium. The nutrition of immature stages and adults of the egg parasite, *Trichogramma* spp. was found to influence the longevity and reproduction. The eggs of *Corcyra* reared on groundnut kernels had appreciable quantities of growth promoting amino acids like glysine, analine and tyrosine and this influenced the size of the adult parasites. The higher fecundity of the parasites obtained from the eggs of *Corcyra* reared on greengram was attributed to the maximum level of nutrients. The individuals which emerged from superparasitized eggs showed reduction in mating and fecundity because of the sharing of the nutrients. The adult longevity and fecundity increased when fed with different sugars in the presence of host eggs. Increase in the protein content in adult diet decreased the fecundity.

In the larval parasite, *Sturmiopsis inferens* the qualitative variation in the nutrient contents due to interspecific variation and quantitative variation as influenced by the size of the host influenced the reproductive capacity of the parasite.

Keywords. Sugarcane pests; natural enemies; nutrition; fecundity.

1. Introduction

Though investigations on nutritional aspects have been done well in some phytophagous and parasitic insects, such basic information is scanty with regard to sugarcane pests and their insect natural enemies. The sugarcane entomologists in the past were more concerned with biology, ecology and control of sugarcane pests. But, it is well understood that basic nutritional information is necessary for species of economic importance, like various lepidopteran and hemipteran insects attacking sugarcane, which may demand studies involving the use of defined food, standardised culture or mass rearings. Attempts already made in this direction has resulted in the development of artificial diet for stalk borer, *Chilo auricilius* Ddgn (Varma *et al* 1975; Bhardwaj *et al* 1984; Jaipal *et al* 1986), internode borer, *Chilo sacchariphagus indicus* (Kapur) (Mehta and David 1978), pink borer, *Sesamia inferens* Wlk (Chatterji *et al* 1969; Qureshi *et al* 1975; Lingappa 1978; Easwaramoorthy *et al* 1986) and Sorghum borer, *Chilo partellus* Swinhoe (Dang *et al* 1970; Seshu Reddy and Davies 1978) infesting sugarcane. However, not much information has been generated on the impact of nutrition on reproductive biology of sugarcane pests and their natural enemies. The available information is summarised in this paper.

2. Sugarcane scale insect, *Melanaspis glomerata* (Green)

2.1 *Influence of sugarcane varieties*

The sugarcane varieties show quantitative variation in the chemical composition and

there occurs significant variation in the reducing and non-reducing sugar content. This is found to influence the establishment, development and reproduction of scale insect, *M. glomerata.* Under laboratory conditions, the number of crawlers that successfully reached reproductive stage varied with the variety. While 72·4% of crawlers reached adult stage on variety Co 740, only 17·1% reached adult stage on CoC 671, probably due to the variation in the nutrient contents. On the other hand the females reared on Co 413 produced significantly more number of crawlers compared to females settled on varieties like Co 775, CoA 7602, CoC 671 and Co 975 (table 1).

2.2 *Influence of media*

The sugarcane setts after the establishment of crawlers, when planted on different media affected the development and fecundity of the scale insect. The proportion of crawlers reaching adult stage (table 2) was more when the medium used to support the sugarcane sett was distilled water, followed by mixed medium (silt, sand and farm yard manure in equal proportion) and White's medium (Thorpe 1981). The fecundity of females was more, when they were reared on cane setts planted in White's medium followed by those that developed on cane setts kept in distilled water. The increased fecundity may be attributed to increase in the size of the insect. This may be due to the availability of more amount of essential nutrients for the insects developing on cane setts planted in these media.

3. Egg parasite, *Trichogramma* spp.

Trichogramma spp. are the most important egg parasites of sugarcane borers in India. It is mass reared on the factitious host, *Corcyra cephalonica* St. The success of mass production in the laboratory and also performance in the field depends to a greater extent on the nutrition.

3.1 *Nutrition of immature stages*

Nutrition of the host insect significantly influences the reproductivity capacity of the

Table 1. Survival (%) and fecundity of scale insect on different sugarcane cultivars.

Variety[a]	Survival (%)	Mean fecundity
Co 413	61·4 (51·62)[b]	160·8
Co 740	72·1 (58·08)	125·7
Co 775	31·5 (34·05)	115·9
Co 975	61·4 (51·62)	102·9
Co 62175	44·5 (41·81)	147·1
CoA 7602	44·8 (42·03)	111·8
CoC 671	17·1 (24·44)	107·2
CoR 8001	36·6 (33·63)	159·5
CD at 5%	7·16[c]	9·59[c]

[a]Sugarcane setts planted on mixed medium
[b]Figures in parentheses are transformed values
[c]Significant at 1% level.

Table 2. Survival (%), weight, fecundity and sex ratio of scale insect on sugarcane setts kept in different media.

Medium	Survival(%)	Weight of 25 scale insects(mg)	Mean fecundity	Sex ratio (M:F)
Sand + White's medium	19·3	235·9	127·1	1:1·67
Mixed + White's medium	31·7	301·4	191·5	1:1·38
White's medium	55·4	281·1	225·9	1:0·77
Sand	21·2	223·9	99·7	1:0·81
Mixed medium	56·0	276·2	147·4	1:1·33
Distilled water	61·4	309·2	219·0	1:0·83
	7·3		30·57	

Table 3. Amino acid contents in the diets and eggs (μg/g)

Amino acid	Groundnut	Greengram	Sorghum
Total free (diet)	2160	5620	3200
Total bound (diet)	1,03,600	86,000	62,720
Total free (eggs)	31,800	29,600	27,400

Table 4. Effect of larval diets on the parasites, *T. chilonis* and *T. japonicum*.

	T. chilonis				*T. japonicum*			
	Size of female(μ)				*Size of female*(μ)			
Larval diet	Length	Breadth	Fecundity	Sex ratio	Length	Breadth	Fecundity	Sex ratio
Sorghum	409·2	171·6	14·5	1:1·20	457·6	158·4	13·5	1:2·78
Greengram	466·4	176·0	39·3	1:1·82	501·6	180·4	23·4	1:2·92
Groundnut	448·8	171·6	30·8	1:1·08	484·0	180·4	12·9	1:2·84

parasite (Navarajan Paul 1980). The effect of host nutrition on the longevity, fecundity and sex ratio of *T. chilonis* Ishii (= *T. australicum* Gir.) was studied by Katiyar (1962). He concluded that the parasite was most efficient when bred on eggs of *C. cephalonica* obtained from crushed sorghum mixed with 8% yeast. Later, Navarajan Paul *et al* (1975) made detailed studies on the effect of eggs of *C. cephalonica* bred on sorghum grains, greengram seeds and groundnut kernels on *T. chilonis* and *T. japonicum* Ashm. The size of the host eggs obtained from the different diets varied greatly. Eggs obtained from the adults of *C. cephalonica* reared on groundnut diet were comparatively larger than those from greengram and sorghum diets. It was noticed that the development of the parasites was influenced by the size and nutrient content of host eggs. Seshagiri Rao (1954) also observed a similar phenomenon in the pupae of *C. cephalonica* reared on different diets.

The eggs of *Corcyra* reared on groundnut kernels had appreciable quantities of growth promoting amino acids like glysine, alanine and tyrosine, when compared with the amino acid contents in the eggs of moths reared on other diets (table 3). This has influenced the size of the adult parasites. In both the species, the size of the adult parasites emerged from the eggs of *Corcyra* reared on groundnut kernels was the biggest (table 4).

The fecundity of the parasites obtained from the eggs of *Corcyra* bred on different diets showed remarkable differences. Highest fecundity of the parasites was noticed

Table 5. Nutrient contents in the eggs of *C. cephalonica.*

	Nutrient contents (%)					
Eggs obtained from	Calcium	Potassium	Phosphorus	Total nitrogen	Total soluble carbohydrates	Glycogen
Sorghum diet	1·40	0·90	0·312	2·80	26·67	12·50
Greengram diet	1·65	2·00	0·450	3·08	40·84	19·17
Groundnut diet	1·50	1·00	0·300	2·80	29·17	17·50

in both the species reared on the eggs of *Corcyra* bred on greengram diet (table 4). The higher rate of fecundity was attributed to the maximum level of nutrients viz calcium, phosphorus, total nitrogen, glycogen and total soluble carbohydrates present in the eggs of moths bred on greengram (table 5).

Even though the different larval diets of the host had influence on the size and nutrient content of the eggs of *Corcyra*, these had no effect on the sex ratio in both the species (table 4).

Another aspect of nutrition of immature stage is noticed when superparasitism occurs. As a result of superparasitism, though 1–3 adults of *T. chilonis* emerged from a single host egg (*Corcyra* egg), they were defective with poorly developed wings. Such individuals were inactive and only a few showed a tendency for mating with forms of about their own size. The fecundity of such females that emerged from superparasitized eggs was low (Chacko 1953; Narayanan and Chacko 1957). The reason for the improper development of the parasites and reduced fecundity is attributed to the sharing of the limited amount of food in the host eggs between the developing parasites.

3.2 *Nutrition of adult parasites*

The adults of *T. evanescens* West. fed on dilute honey lived significantly longer and produced more progeny than starved females (Lund 1938). Later, Narayanan and Mookherjee (1955) made detailed studies on adult nutrition. There was a significant increase in the longevity and fecundity of *T. chilonis* adults when fed with 10% solutions of glucose, fructose, maltose and sucrose compared to unfed females. Longevity and fecundity also increased to a certain extent with the addition of 10% solution of yeast extract, *Corcyra* eggs extract or centrifuged milk, to a 10% solution of glucose given as feed in equal proportions (table 6). There was a great increase in the longevity of the parasite, when fed merely on sugar than when fed with different proteins in combination with glucose. The fecundity of the parasite was highest when fed merely on sugar in the presence of host eggs and an addition of yeast extract, *Corcyra* egg extract or skimmed milk to the sugar solution lowered its fecundity. So, it is evident that host proteins are of more value than other proteins. The parasite, however, seems to need only a limited quantity of the host protein to lay its normal quota of eggs and any increase in the proportion of its protein beyond what the parasite normally imbibes from eggs at the time of oviposition has resulted in a lowering of its fecundity. It is, therefore, apparent that its protein requirements are normally derived from the host itself.

Ramachandra Rao (1955) also reported that the fecundity and longevity of

Table 6. Effect of adult nutrition on longevity and fecundity in *T. chilonis*.

Treatments	Longevity of males (days)			Longevity of females (days)			Fecundity of females*
	No mating Host eggs not provided	Mating Host eggs not provided	Mating Host eggs provided	No mating Host eggs not provided	Mating Host eggs provided	Mating in presence of host eggs and laying eggs	Number of *Corcyra* eggs parasitised
No food	0·99	1·15	1·06	1·49	1·21	1·80	46·05
Glucose	4·00	4·30	4·80	18·40	10·15	13·50	94·90
Maltose	3·95	3·65	4·75	9·20	7·35	11·45	94·40
Fructose	4·95	4·95	5·15	10·10	7·90	14·05	106·60
Sucrose	5·65	4·15	4·65	9·25	8·70	10·80	81·30
Skimmed milk and glucose	3·95	1·60	1·95	3·70	2·20	3·45	57·90
Yeast extract and glucose	3·30	2·75	2·55	5·70	4·00	5·55	69·45
Corcyra eggs extract and glucose	3·70	2·80	2·40	7·60	3·45	7·65	69·45
Tap water	2·25	1·27	1·23	2·57	1·14	1·76	35·1
Corcyra egg extract	1·67	—	—	1·27	—	—	—
CD at 5%	0·86	1·07	1·40	1·95	1·57	0·96	20·50

*Females mated and laid eggs in the presence of host eggs.

Table 7. Effect of different hosts on fecundity of *S. inferens*.

Host	Mean host pupal weight (mg)	Mean parasite puparial weight (mg)	Mean fecundity (number/female)
Chilo infuscatellus Snell.	72·7	34·0	196·4
Chilo partellus Swinhoe	79·4	40·8	252·6
Scirpophaga excerptalis Wlk.	96·0	39·3	270·0
Sesamia inferens Wlk.	123·3	48·5	322·0
Galleria mellonella (L.)	115·6	41·3	357·1
Corcyra cephalonica Stn.	23·6	21·2	No mating occurred

T. chilonis increased considerably when the parasites were fed on 10% solution of different sugars. Both virgin and mated females fed on honey-water solution lived upto 5 days and retained their eggs upto 72 h after emergence without any detrimental effect (Sharma 1968).

4. Larval parasite *Sturmiopsis inferens* Tns.

S. inferens is an endo-larval parasite of sugarcane shoot borer, pink borer, stalk borer and Gurdaspur borer, *Acigona steniellus* Hmpsn. Though the exact nutrient requirements of the parasite was not worked out, it was found that the size of the host is found to influence the size and fecundity of the parasite (table 7). A positive correlation was obtained between the weight of the host pupae and parasite puparia irrespective of the host. The weight of the puparia, in turn, influenced the fecundity of

Table 8. Influence of host sex on the pupal and adult weight.

	Weight (mg)					
	Male parasite	Female parasite	Mean	Male parasite	Female parasite	Mean
	Male host			Female host		
Host pupa	44·2	43·0	43·6	86·0	87·2	86·7
Parasite puparia	25·0	24·8	24·9	39·3	38·7	38·9
Parasite adult	18·1	17·1	17·8	23·8	23·9	23·8
	Host pupa		Parasite puparium		Parasite adult	
	SE	CD	SE	CD	SE	CD
Between host sex	2·44**	5·48	2·04**	4·58	1·02**	2·81
Between parasite sex	NS		NS		NS	

the adults (David *et al* 1980). So, the qualitative variation in the nutrient conter due to interspecific variation and quantitative variation as influenced by the size the host affected the reproductive capacity of the parasite.

Even within a host, the quantitative variation in nutrition, as influenced by the si of the host had an effect on the parasite weight and fecundity. The data presented the weight of shoot borer pupa, parasite puparia and adult in table 8 reveal that the puparia and adult parasite developed from female pupae of the ho *C. infuscatellus* were bigger irrespective of the sex, than that developed from ma pupae. Again weight of the adult parasite had a positive association with fecund ($r = 0{\cdot}3824^*$).

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Nutrition and reproduction in haematophagous arthropods

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Abstract. Hosts of haematophagous arthropods range from amphibians to mammals. Blood meal is essential for egg production. Quantity and quality of ingested blood are important in realization of optimum reproductive potentials. In terms of egg production, the lowest nutritive value is for human blood. This inequality is probably based on differences in proteins and their constituent amino acids. Carbohydrates in the diet have no direct contribution to reproduction. Very little is known about sterol, lipid, vitamin and salt requirements for reproduction. In the mosquito *Aedes aegypti* some substance from the blood meal is thought to initiate vitellogenesis and is sustained by a complex hormonal mechanism in which median neurosecretory cells, corpus allatum, fat body and ovary participate. Mechanical stimuli from gut distension also has a role in hormone induction.

Autogenous egg production seen in many dipterans is controlled both by the nutrition of the immature stages and genetic mechanisms.

Quantity and the rate of utilization of the ingested blood decide the frequency of host visitation and feeding. Vertebrate blood sera contain substances which stimulate (secretogogue) as well as inhibit digestion. Nutrition plays some role in male maturation in insects, though not in spermatogenesis. In many ticks even spermatogenesis is influenced by adult blood meal.

Keywords. Haematophagous arthropods; insects; nutrition; reproduction; blood meal; autogeny; nutrient metabolism; host visitation.

1. Introduction

Among haematophagous endopterygotes only the adults are parasitic whereas haematophagous exopterygotes, often spend their entire life cycle on the host. Examples of the former are Diptera (Nematocera, Brachycera and Cyclorrhapha) and Siphonaptera. The latter has Anoplura and Hemiptera. Except in cases where autogenous egg production is seen (e.g. certain spp. of mosquitoes, *Culicoides*, *Phlebotomus*, simuliids etc) blood meal is essential for ovarian maturation in all haematophagous endo and exopterygotes. Even in those which are autogenous, blood meal is necessary to mature the second and subsequent batches of eggs. Typical examples of haematophagous nematocerans are the Culicidae (mosquitoes); Ceratopogonidae (biting midges); Simuliidae (black flies) and Psychodidae (sand flies). Among mosquitoes, except in autogenous forms, blood meal is essential for egg production and they feed on a variety of hosts ranging from amphibians to mammals. *Culicoides* (Ceratopogonidae) and simuliids feed on birds and mammals, while psychodids have a wide range of hosts from amphibians to mammals. Nectar feeding is a common feature in both the sexes (Downes 1958; Lewis and Domoney 1966). The only large group of Brachycera which habitually suck blood is those belonging to the family Tabanidae feeding mostly on mammals. Like Nematocera feeding dichotomy is seen, both sexes feeding on honey dew or nectar. Only females are haematophagous and blood meal appears necessary for ovarian maturation and egg production (Wilson 1967). According to Downes (1958) nectar feeding in

tabanids is a secondary development these being originally obligate blood suckers. Stomoxynae, Glossinidae and Pupipara (the louse flies Hippoboscidae, batflies Nycteribidae and Streblidae) are important groups of blood feeding Cyclorrhapha. Vertebrate blood is the only food taken by both sexes. *Glossina* feeds mainly on mammals whereas Pupipara have birds and mammals as hosts. *Stomoxys* and *Haematobia* are anautogenous blood suckers feeding mainly on ungulates and requiring several blood meals to complete ovarian development. Both sexes of the order Siphonaptera are obligate blood feeders parasitizing birds and mammals. Though Pthiraptera as a rule are obligate ectoparasites of birds and mammals, habitual haematophagy is found only among Anoplura (sucking lice) and in these cases blood is the only food. Hemiptera is a large assemblage exhibiting both phytophagy and zoophagy. Obligate haematophagy is exhibited by certain members of Reduviidae and Cimicidae. In these cases both sexes feed on blood and vertebrate blood is the only food. Cimicids feed on both birds and mammals. Polyctenids are exclusive parasites of bats. Triatominae of Reduviidae feed exclusively on vertebrate blood using mammals as hosts. Ticks are all obligate blood suckers at all stages of their life cycle, having a wide variety of hosts ranging from reptiles to mammals. Autogeny is reported in some ticks, but barring these, blood meal is essential for reproduction and a critical minimum amount of blood is needed for oviposition (Oliver 1974). Members belonging to families Psoroptidae, Dermanyssidae and Macronyssidae are probably the only haematophagous mites (DeLoach and DeVaney 1981; DeLoach and Wright 1981; Wright and DeLoach 1980, 1981).

One gonotrophic cycle may be said to consist of 3 phases: (i) locating a host and feeding on it; (ii) digestion of the blood meal and egg production and (iii) search for suitable oviposition site and egg laying (Detinova 1962). This review is focussed mainly on the second phase.

2. Blood meal and reproduction

Both quantity and quality of the blood meal influence the reproductive potentials of haematophagous insects. The size of blood meal influences not only the fecundity, but acquisition and transmission of pathogens (Hovanitz 1947; Jeffrey 1956), midgut protease activity (Shambaugh 1954) and refeeding behaviour (Edman *et al* 1975; Klowden and Lea 1978) as well. The number of blood meals required (related to the quantitative requirement?) to lay full complement of mature eggs varies from one to several. Though gonotrophic concordance [a condition where there is a strict alternation of blood feeding and oviposition (Kettle 1984)] may be seen in several blood sucking Diptera including certain mosquitoes, simuliids, tabanids and phlebotomines, certain others are shown to require more than one blood meal to complete one gonotrophic cycle (Beach *et al* 1983; Corbet and Smith 1974; Detinova 1962; Gillies 1954, 1955; Killick-Kendric *et al* 1977; Rao 1947; Thomas and Gooding 1976). The number of pre-ovipositional blood meals required by several floodwater mosquitoes like *Aedes vexans*, *Psorophora confinnis* and *Psorophora cyanescens* vary from 1–14 (Breeland and Pickard 1963, 1964; Chapman and Woodard 1965). Larval/nymphal nutrition could have a role in deciding the number of pre-ovipositional blood meal required by the adult. MacDonald (1956) showed that female *Aedes* from well fed larvae needed only one pre-ovipositional blood meal to lay one raft of eggs

whereas those from poorly fed larvae had to feed twice before laying eggs. There is a close correlation between quantity of blood ingested and the number of eggs laid on the one hand and degree of host association on the other. Those which take small meals (usually less than their own body weight) at frequent intervals to lay small batches of eggs are more closely associated with the host, example *Haematobia irritans* (Diptera) and *Xenopsylla cheopis* and *X. astia* (Siphonaptera). Oviposition in these cases, occurs daily and they would always have some follicles in the process of maturation. Gravid females ready for laying would have 2nd set of follicles already well advanced in vitellogenesis. In other words once initiated, vitellogenesis in these cases is a continuum until the life-time egg production potential is reached (Krafsur and Ernst 1983; R S Prasad, unpublished observations). Mosquitoes which require only one blood meal to lay a full complement of eggs of one batch (a full batch of eggs is one egg/ovariole and the number is limited by the number of ovarioles) ingest blood 1 to $1\frac{1}{2}$ times their own body weight and lay eggs numbering 100–150. In these cases ingestion of suboptimal quantity of blood does not result in oviposition. For example no ovarian development was seen in *Anopheles maculipennis* which took incomplete blood meal (Detinova 1962).

Ticks which ingest large meal (about 120 times their own body weight) lay thousands of eggs. The quantity of the reserve yolk packed into individual egg (size of the egg) and the amount of nutrient available to the system are important aspects to be reckoned with this type of correlative studies. For example, *Rhodnius* which takes a very heavy blood meal compared to lice or fleas lays on an average 200–300 eggs in its life time of 4–5 months, whereas probably a similar number or more is laid by a female of *Pediculus humanus* or *X. cheopis* during its life time of a little over a month. But then the egg of *Rhodnius* is 3–5 times larger than *Pediculus* or *Xenopsylla*. The same or larger number of eggs laid during a shorter period suggest that the interphase between two layings in *Rhodnius* must be more protracted than the other two. The possibility that this is an adaptation for slower digestion of a larger meal and more efficient utilization of the nutrient content would be a good guess. What exactly is the correlation (if there is any) between the weight of the blood meal and that of the egg is not known. That there is considerable interspecific variation is evidenced by the findings of Goodchild (1955) who showed that the minimum amount of blood which allowed some eggs to mature was about 51 mg in *Rhodnius*, 149 mg in *Triatoma* and according to Colles and Chellappa (1960) and Spielman and Wong (1974) 0·4–0·5 mg in *Aedes aegypti*. Several insects when given a chance to ingest more quantity of blood would lay more eggs upto a limit permitted by the number of ovarioles (Kamala Bai and Prasad 1981; Khalifa 1952).

Reports show that blood from different vertebrates influence fecundity rates of haematophagous insects. Human blood has a poor nutritive value compared to blood from several other vertebrates, for *Culex pipiens* (Tate and Vincent 1936; Woke 1937b), *Cx. p. berbericus* and *Ae. aegypti* (Woke 1937a), *Cimex lectularius* (Johnson 1942) and the fox strain of the flea *Pulex simulans* (Hudson and Prince 1958). Judson (1986) found the critical period (the interval following blood meal when the head of a female mosquito must be present for initiation of follicular development leading to maturation) of *Ae. aegypti* females fed on guinea-pig blood was considerably shorter than those fed on human blood. Despite its higher protein content human blood has the lowest efficiency in protein utilization and incorporation of nitrogen into egg. Total nitrogen excretion after ingesting human blood was

78% whereas with guinea-pig blood it was 53% (Briegel 1985). On the other hand *Simulium underhilli* produced fewer eggs from rat blood offered as enema than they did from equal volume of human blood (Klowden and Lea 1979c). Ikeshoji (1965) showed that *Cx. pipiens* laid more eggs when fed on avian host. The fecundity rate of *Culiseta melanura* was more on blood meals from chicken than from rabbit (Nasci and Edman 1981) when 3 μl blood meals were administered by enema. This suggests a physiological adaptation to avian hosts. Several similar studies have indicated that mammalian blood has a low nutrient value for mosquitoes compared to blood from amphibians, reptiles and birds. Fecundity and longevity of *Cimex hemipterus* was greatly reduced in those fed on blood of *Calotes versicolor*, compared to blood from man, rat and fowl (Sreelatha 1984). However, Nayar and Sauerman (1977) showed that though egg yield of *Anopheles quadrimaculatus*, *Ae. aegypti*, *Culex nigripalpus* and *Psorophora columbiae* fed on chicken blood was more than those fed on human blood (18–38% more oocytes were developed on chicken blood) in general on a per mg basis avian blood did not promote increased egg production in these mosquitoes. The reason for this kind of nutritional inequalities of blood from different vertebrate hosts could be many. Several artificial feeding experiments have pointers to blood proteins as the first suspect. As early as 1938, Yoeli and Mer, based on their experiments on *Anopheles elutus* suggested protein to be the factor which trigger egg development. Spielman and Wong (1974) found that enemas of 2·0 mg or more serum resulted in oocyte maturation and that the defibrinated human blood, washed cell components, serum alone and bovine albumin and globulin were adequate to stimulate oogenesis in *Ae. aegypti*. Unlike the mosquito *Ae. aegypti* (Woke 1937b) rat fleas *X. cheopis* and *X. astia* could not mature their ovaries on rat blood plasma diet, but the blood cell fraction was nutritionally adequate to trigger oocyte maturation. For these rat fleas washed blood cells resuspended in normal saline was an adequate diet to trigger vitellogenesis whereas blood plasma alone was found inadequate. Addition of blood-cell-bound-substances like ATP, serotonin and glutathione to plasma did not make it adequate diet for initiation of ovarian maturation, but reinforcing plasma with rabbit albumin thereby increasing its protein concentration made it an adequate diet for triggering vitellogenesis. If a gonoactive female of rat flea is put on a plasma diet, extensive oocyte resorption could be seen. These findings indicate that dietary protein concentration is a decisive factor for vitellogenesis (Kamala Bai and Prasad 1976). Similarly over 2 dilutions of rat blood with physiological saline inhibited vitellogenesis (Bella 1978). *Glossina palpalis* requires serum albumin for norml digestion of RBC and normal fecundity (Takken 1980).

Insects show specificities to protein species for egg development. For example, in their study Lea *et al* (1956) found that only certain protein or their enzyme hydrolyzates stimulate egg production in *Ae. aegypti*. Lactalbumin was found to be superior for the mosquito *Aedes taeniorhynchus* (Nayar 1966) whereas purified egg albumin was found to be a better protein source for several other mosquitoes (Lang *et al* 1972). Rat fleas *X. cheopis* and *X. astia* were not able to utilize bovine albumin in place of bovine haemoglobin in an artificial diet for yolk synthesis (Kamala Bai and Prasad 1979a). Greenberg (1951) found that for *Ae. aegypti* bovine plasma albumin, casein, gelatin or globulin had a superior nutritive value than erythrocytes (haemoglobin) with regard to egg production.

The biological (nutritional) value of any protein is a function of its amino acid composition. Digestibility and proportion of complement of amino acid it provides

govern the adequacy of a protein. Greenberg (1951) found that addition of isoleucine to a variety of dietary proteins improved egg laying in *Ae. aegypti*. Additions of amino acids to a suboptimal mixture of essential amino acids promoted greater egg production in *Ae. aegypti* (Lea *et al* 1956). *Ae. aegypti* requires 8 essential amino acids (arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine) plus histidine, methionine and cystine are required in the diet for optimum egg production. Deletion of the 8 essential amino acids resulted in no egg production while the deletion of the other 3 caused lesser number of eggs to be produced. For optimum egg production, it is not only the presence of these 11 amino acids, but the proper balance between the amino acids in the mixture was also important (Dimond *et al* 1956). Attempts to replace protein with amino acids in an artificial diet for fleas *X. cheopis* and *X. astia* failed (Bella 1978). Amino acids present in the diet should be the utilizable isomer. For example D-isomers of isoleucine, threonine and valine are not metabolised and so inhibit egg production. The principle of nutrient proportionality suggests that metabolically suitable proportions of nutrients are needed for normal nutrition. Disproportionately abnormal amounts of even a single amino acid can reduce the efficiency in converting food stuff and can inhibit feeding and growth. Balance of nutrients can be one of the factors that determine choice of a food material (House 1974). If a single essential amino acid is absent from the diet or present in suboptimal levels, the amino acid in question rapidly disappears from the haemolymph, others increase in amount, protein synthesis comes to a halt and uric acid excretion rises rapidly (Dadd 1985).

The utility of carbohydrates in the diet has been fairly well established in the case of mosquitoes and certain other dipterans. Sugar meal enhances longevity and supplies energy needed for flight activity in many blood sucking dipterans (Corbet 1964; Davies 1953; De Meillon *et al* 1967; Lee and Davies 1979; Lewis 1953). Clements (1955) showed that subsequent to sugar ingestion there is accumulation of fat and glycogen. Correlation between glucose absorption and synthesis of glycogen and triglycerides was shown by Van Handel (1965). Glycogen is used as a source of energy mainly for flight while triglycerides for general maintenance functions such as respiration, excretion, digestion etc (Nayar and Van Handel 1971). In the case of males only small quantities of triglycerides are synthesized from sugar (Van Handel and Lum 1961). According to Nayar and Sauerman (1975a) glucose absorption, triglyceride accumulation and depletion are faster in *Ae. aegypti* and *An. quadrimaculatus* and the duration of survival shorter. In *Aedes sollicitans* and *Ae. taeniorhynchus*, on the other hand, these processes are slower and survival (longevity) longer. Sugar does not appear to have any direct role in egg production in mosquitoes (Lea *et al* 1956), but it does influence egg production. Magnarelli and Anderson (1981) showed fructose and sucrose as essential dietary nutrients for *Chrysops* sp., *Hybomitra* sp. and *Tabanus* sp. which feed extensively on sugars. They also suggested that some sugars may be converted and deposited as yolk during early oocyte development, but compared to vertebrate blood, these supplementary carbohydrates probably have little importance in the vitellogenic process of anautogenous tabanids. Hecht (1933, cited by House 1958) found carbohydrates beneficial for reproduction of *An. maculipennis* at suboptimal temperature but had no effect at optimum temperature. It is possible that variation in metabolic rates of insects would have corresponding differences in dietary needs of nutrients. There are only 3 known cases (*Culex quinquefasciatus*, *Aedes atropalpus* and *Ae. vexans*) of carbohydrate diet inhibiting oviposition (De

Meillon *et al* 1967; Hudson 1970; Shroyer and Sanders 1977). De Meillon *et al* (1967) found that by the 5th day after the blood meal only 45% of the sugar fed *Cx. p. quinquefasciatus* laid eggs compared to the 98% in the sugar free group. Carbohydrate starved *Ae. vexans* laid 49 eggs/oviposition whereas females allowed to ingest sucrose deposited approximately 22 eggs/oviposition (Shroyer and Sanders 1977). As has been pointed out by these authors carbohydrate-dependent delay in oviposition and the attendant increased longevity could have adaptive values when oviposition sites are difficult to find during certain periods or for spp. which utilize temporary breeding sites. However in yet others sugar feed prior to blood meal resulted in laying more eggs and substantially longer survival than starved female mosquitoes. The latter was also shown to require more wet weight of blood to lay 100 eggs/female (ranged from 9·3–15·7 mg in different spp) compared to the former (sugar fed) group (ranged from 3·4–5·2 mg in different spp) (Nayar and Sauerman 1975b). Lee and Davies (1979) suggested that in stablefly *Stomoxys calcitrans* sugar meal before having the first blood meal appears important in the general well being of the flies. Digestion of blood meal was delayed when a 10% sugar solution was also fed compared to those which fed on blood alone. Haematophagous insects appear to compensate for the low dextrose level of blood by resorting to feeding on plant nectars (Dubose 1975, cited by Fritz 1983). Very little information is available on those which feed exclusively on blood. For streblids which feed exclusively on blood, frequent feedings appear to provide the required sugars for adults. If these insects are removed from their hosts for long periods of time they may be literally starved to death. Streblids may be used in nitrogen for both longevity and reproduction (Fritz 1983). Deletion of sugars from the basal holidic diet of rat fleas *X. cheopis* and *X. astia* resulted in failure of yolk deposition in the former species, but about 13% of the latter showed yolk deposition. This interspecific difference may be linked up with larval nutrition as small percentage of *X. astia* could mature their ovaries in the absence of vitamins, salts, sugars and cholesterol (Kamala Bai and Prasad 1979a). All the carbohydrates are not equally utilized. Survival of adult *Ae. aegypti* was good on glucose, fructose, sucrose, maltose, raffinose etc., but poor on arabinose, cellobiose etc (Galun and Fraenkel 1957).

Rather poorly known are the requirements of sterols, lipids, vitamins and salts of haematophagous insects for reproduction. An interesting example of sterol requirement for reproduction is that of the rabbit fleas *Spilopsyllus cuniculi* and the hare flea *Cediopsylla simplex*. Rothschild and her colleagues did extensive studies on the reproduction of *S. cuniculi* and discovered that they could breed only when the blood level of corticosteroids of their host rabbit increases. They also found that the natural condition can be stimulated by external application (spraying) of hydrocortisone on the fleas. Progesterone induced ovarian regression in the rabbit fleas (Rothschild and Ford 1964a,b; 1966). However, reproduction of rat fleas *X. cheopis* and *X. astia* are not under the control of host's hormones (Prasad 1969, 1973, 1976). Deletion of cholesterol from an artificial diet for these rat fleas resulted in no yolk deposition (Kamala Bai and Prasad 1979a). Dimond *et al* (1958) found sterol having no influence on egg production of *Ae. aegypti*. Whether this sterol independence is the result of microbial action or not is not known. Lipid associated with serum proteins are important in nutrition and reproduction of tsetse fly *Glossina morsitans*. Flies fed on delipidated serum or delipidated albumin failed to reproduce. There was an apparent correlation between lipid content of the different albumins and the size of

the offsprings produced by the flies fed on them (Kabayo 1982). *Ae. aegypti* can produce eggs in lipid-free diets (Dimond *et al* 1956). According to House (1958) it is unlikely that the adults are dependent on dietary lipids because lipids can be synthesized from carbohydrates and proteins. Recently it was found that arachidonic acid or certain structurally related long-chain polyunsaturated fatty acid appears to be a general mosquito requirement and must be present in the larval diet for production of viable adult *Cx. pipiens* (Dadd 1981; Dadd and Kleinjan 1978, 1979; Sneller and Dadd 1981). Arachidonic acid, a precursor of prostaglandin E2 (PGE2) are known to influence reproduction also (Dadd 1985). Dimond *et al* (1958) found no stimulation of egg production upon addition of vitamins and nucleic acids. Nayar and Sauerman (1977) concluded that nucleated RBC of amphibians, reptiles and birds did not confer any additional nutritive quality. Feeding the bed bug *C. lectularius* on folic acid deficient host resulted in reduced egg production. De Meillon and Golberg (1946, 1947a,b) found considerable reduction in egg production of the tick *Ornithodoros moubata* fed on thiamine deficient rats. However, Wanchinga and Sonenshine (1978) found no effect on the tick *Amblyomma maculatus* fed on thiamin deficient rats. The major difficulty in interpreting this type of experiment is to draw a direct relation between deficiency and egg production. Vitamin deficiency could have several other secondary effects on the systems of the host such as for example accumulation of pyruvate in the blood due to thiamine deficiency and which could be toxic to the blood sucking insect (Nelson 1984). Pillai and Madhukar (1969) found that adult *Ae. aegypti* emerging from biotin fed larvae showed follicular degeneration during postovipositional period and suggested that biotin interferes with certain metabolic processes of egg maturation. They also showed effect of biotin to be specific to ovary and not to testis. Vertebrate blood is deficient in B-vitamins. For those insects which subsist only on vertebrate blood, symbiotic microorganisms mostly concentrated in mycetomes in different parts of the abdomen including gut (Hippoboscid, Nycteribiid, Glossina, Cimicid, Reduviid and Anoplura) supply the necessary B-vitamins (Brookes 1964). Dimond *et al* (1958) found the presence of sodium and potassium in the diet increased egg production of *Ae. aegypti. Cx. pipiens* could lay eggs when fed on milk or on plant proteins, if Fe ions are present (Huff 1929; De Boissezon 1933). Wanchinga and Sonenshine (1978) found calcium deficiency in rats caused the ticks *A. maculatus* to lay fewer eggs and in these egg hatchability was reduced. High concentration of calcium in the diet of rabbits was toxic for *C. lectularius* (De Meillon *et al* 1948). This mineral appeared to interfere with water excretion (Nelson 1984). De Meillon and Golberg (1947b) and De Meillon *et al* (1947) found no effect on egg production when *C. lectularius* and the tick *O. moubata* were fed on riboflavin deficient rats.

3. Physiological aspects of nutrient influence on reproduction

Engelmann (1970) suggested several pathways by which nutrition or nutritional factors may affect egg maturation. According to his scheme (p. 128), on the one hand food can directly influence egg maturation by supplying either the raw materials or food factors essential for the synthesis of yolk. On the other, food can have an indirect influence on yolk synthesis via the central nervous/endocrine systems. With regard to haematophagous insects blood proteins are factors without which sus-

tained oocyte development becomes impossible. Regression of developed oocytes when gonoactive female rat fleas were put on rat blood plasma diet which is sub-optimal for protein requirement is one of the examples (Kamala Bai and Prasad 1976).

Gut distension as a result of feeding (mechanical stimuli) appears to play a role in the egg maturation of certain haematophagous arthropods like the mosquitoes *An. maculipennis* (Detinova 1953), *Cx. pipiens* (Larsen and Bodenstein 1959) and the tick *O. tholozani* (Gabby and Warburg 1976). In the two species of mosquitoes ingestion of a non-proteinaceous meal after anal occlusion resulted in initiation of egg maturation. Activation of corpora allata via the nervous system is suggested. However, in rat fleas *X. cheopis* and *X. astia* the role of gut distension is questionable. These fleas require more than one blood meal for the induction of yolk deposition. Milk (whole cow milk/milk diluted with normal saline) failed to induce yolk deposition. These fleas also feed on blood plasma if ATP is added, but the low protein concentration of this diet failed to induce yolk deposition (Kamala Bai and Prasad 1976). It is probably the haemolymph protein concentration or certain particular blood fractions (some peptides/amino acids?) which trigger yolk deposition. Total haemolymph proteins increased by almost 2 times after feeding rat fleas *X. cheopis* and *X. astia* on a live rat. Whereas in fleas membrane-fed on plasma or sucrose, total haemolymph protein remained at unfed teneral level and showed no increase whereas sucrose fed fleas showed a very slight increase in the total haemolymph proteins. Fleas membrane-fed on whole blood of white rat and those fed on live rats showed almost comparable levels (slightly less in the former) of total haemolymph proteins (Narayana Pillai and Prasad 1986).

As the endocrine influence is not the subject theme of this essay the reader is referred to excellent accounts on the influence of hormones on reproduction by Clements (1963), Engelmann (1970) and Hagedorn (1985). Much of the existing information on endocrine control of reproduction of haematophagous insects come from the studies on mosquitoes. As early as 1936, Wigglesworth demonstrated through decapitation and parabiosis experiments that corpora allata are necessary for egg maturation in *Rhodnius prolixus*. He also demonstrated a critical time after which removal of corpora allata did not inhibit egg maturation. Later studies have shown this finding to be true for mosquitoes also. Though working with *Ae. taeniorhynchus*, *Ae. aegypti*, *Ae. sollicitans* and *Aedes triseriatus*, Lea (1963) showed that allatectomy had varied effects on egg development within a single genus *Aedes* and even within one species, in general both autogenous and anautogenous mosquitoes need hormones from the brain and corpora allata for egg maturation (Lea 1967). Lea (1970) showed that in autogenous forms the hormones were in circulation even without a blood meal while in anautogenous forms only the corpus allatum hormone was in circulation before a blood meal. Median neurosecretory cell (MNC) hormone accumulates in the corpora cardiaca after emergence and it is released into haemolymph only after a blood meal. The existing information on hormonal control of oogenesis in mosquitoes may be summed up as follows. Two stages in oogenesis can be recognised. The 1st stage involves the previtellogenic development leading to resting stage after emergence in which they remain until after a blood meal. This stage is mediated by juvenile hormone from corpora allata (Gwadz and Spielman 1973). In the second stage, blood meal triggers the release of a hormone from the MNC [egg development neuro-hormone (EDNH) described by Lea 1972] which in

turn stimulate the ovaries to synthesize and release ecdysone. The ovarian ecdysone released into the haemolymph gets converted into 20-hydroxyecdysone (20-HE) in the fat body and triggers vitellogenin synthesis by the fat body cells (Hagedorn 1974, 1985) and separation of the secondary follicles. Beckemeyer and Lea (1980) in *Ae. aegypti* and Redfern (1982) in *Anopheles stephensi* have shown that the presence of 20-HE is necessary to stimulate the separation of a new follicle from the germarium. The general scheme on the action of hormones on gonotrophic cycles of anautogenous mosquitoes is given by Hagedorn (1985). In this scheme the process of egg maturation in mosquito is shown to require the sequential appearance of juvenile hormone (JH), EDNH and ecdysone for each gonotrophic cycle. JH has a direct stimulatory effect on vitellogenin synthesis even when the JH titer is declining, but this effect is seen only in the presence of 20-HE (Hagedorn 1986). Blood meal triggers the synthesis of both messenger and ribosomal RNA necessary for vitellogenin synthesis in *Ae. aegypti* (Hagedorn *et al* 1973).

Van Handel and Lea (1984) showed that neither corpora allata nor the cerebral neurosecretory system, including the corpus cardiacum nor any ovarian factor is required to induce synthesis of vitellogenin in *Ae. aegypti.* However, continuous synthesis of vitellogenin depends on factors from both the head and ovaries. It is concluded that blood meal itself and not stretching of the gut is the stimulus needed to initiate vitellogenin synthesis and that a digestion product of blood either stimulates the fat body directly or some of the endocrine-like cells in the midgut which serve as transducers between the blood meal and fat body. Nayar and Sauerman (1977) working on *Ae. aegypti, Cx. nigripalpus* and *Ps. columbiae* found that blood clotted in the gut in 30 min and absorption of the separated serum components resulted in immediate initiation of oogenesis, but promotion of initiated oocytes followed the digestion of the coagulated blood. In the stable flies *S. calcitrans* sex pheromone secretion is under dietary control. Comparing blood or glucose-fed *S. calcitrans* Meola *et al* (1977) showed that only blood diet induced sexual behaviour and that sex pheromone (cuticular hydrocarbons) are produced only in the blood fed group.

In *Ae. aegypti* JH level is high before feeding but declines after feeding. Meola and Petralia (1980) showed that JH influences blood feeding activity of *Cx. pipiens* and *Cx. quinquefasciatus.* JH deprivation caused by surgical removal of corpora allata shortly after adult emergence blocked the initiation of biting behaviour in these mosquitoes.

4. Autogeny and nutrition

Autogeny is defined as capacity to develop and lay eggs without a proteinaceous meal. Several haematophagous Diptera among others exhibit autogeny (Spielman 1971). Since in such cases the adults lay eggs without a blood meal, the major source of yolk must be derived from the reserve nutrients carried over from the larval stages (Briegel 1969; Clements 1956; Spielman 1957). Twohy and Rozeboom (1957) found higher rates of lipids and glycogen in the larvae and pupae of the autogenous *Culex molestus.* Autogenous mosquitoes contain large stores of nutrient than anautogenous mosquitoes (Clements 1956; Lang 1963). Deficiencies in the larval diet can also prevent expression of autogeny in mosquitoes (O'Meara and Evans 1976).

Chen (1967) during his electrophoretic studies on larval haemolymph of autogenous and anautogenous populations of *Cx. pipiens* discovered a protein fraction present in the former but not in the latter and suggested this to be involved in the endocrine control of autogeny. However, Spielman (1971) does not contribute to this idea. Lea (1964) found that the expression of autogeny in the highly autogenous strain of *Ae. taeniorhynchus* may be reduced by altering the quality and quantity of larval diet and withholding sugar from newly emerged females.

Allatectomy within 1 h after emergence prevented autogenous yolk deposition in *Ae. taeniorhynchus* (Lea 1963). Larsen and Bodenstein's (1959) conclusion that in anautogenous forms corpora allata were inactive until activated by a blood meal whereas corpora allata of the autogenous females were active soon after emergence without the necessity for blood feeding is not supported by the findings of Lea (1963, 1969) who showed that corpora allata of anautogenous female were active prior to the blood meal. Van Handel (1976) showed that the main source of yolk protein in the autogenous *Aedes atropalpus* is not the flight muscle but fat body itself. The contribution to yolk protein synthesis is negligible. Flight muscle may form a potential source of nutrient for autogenous production of yolk as has been suggested by Hocking (1954) in the case of non-haematophagous mosquito *Aedes communis*. This, however, is not the case in *Ae. atropalpus*. According to O'Meara and Evans (1977) the salt marsh mosquito *Ae. taeniorhynchus* has two types of autogenous females. One that requires a stimulus from mating and the other that does not. A substance from the male accessory glands (matrone?) appear to stimulate autogenous egg production. Autogeny, however, is also under polygenic control (Spielman 1957, 1971). Autogenous egg production can be viewed as an insurance against unfavourable situations.

5. Nutrient metabolism and host visitation/feeding

One gonotrophic cycle, bounded by two host seeking activities and feeding, is designated in the present context as an event of feeding and nutrient utilization culminating in oviposition. Frequency of feeding in the case of haematophagous insects, irrespective of whether they are permanent residents on the body of the host or spend short time on the host is controlled by the quantity of blood ingested and the pace of utilization of the ingested nutrient. In other words rate of utilization of the ingested nutrient or that which is already stored decides the frequency of host visitation and feeding.

Factors which can regulate the pace of host visitation, feeding and utilization of ingested blood are outlined in figure 1. Obviously the first step in utilization would be the rate of digestion as most of the dietary components are to be first broken down before absorption and utilization for house keeping metabolic functions and reproduction. Permeability of the insect intestine to large peptides is restricted (Turunen 1985). Time required for oogenesis and blood meal digestion are interrelated in most gonoactive haematophagous insects and duration of oogenesis establishes the minimum time between blood meals (Roberts *et al* 1983).

Diet and dietary components appear to have very important role to play in rates of enzyme secretion and digestion. A secretogogue (specific dietary components elicit a direct response in the intestinal cells involved in enzyme synthesis) control of

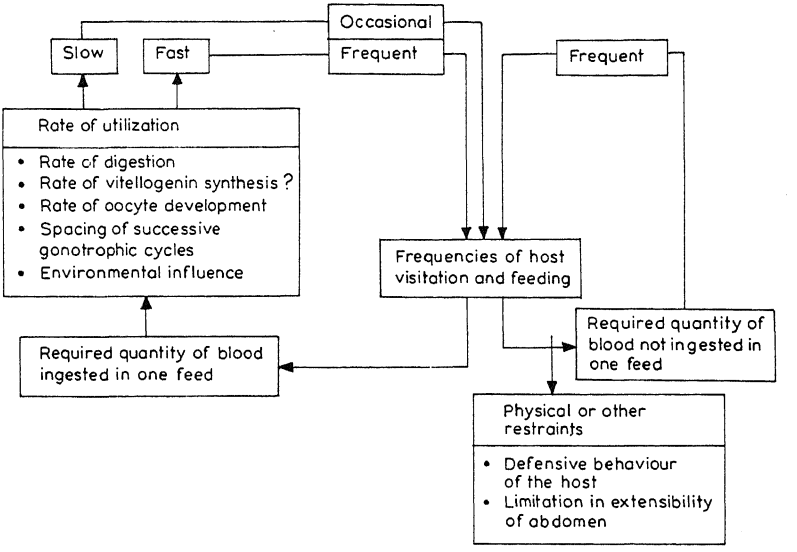
Occasional
Slow
Fast
Frequent
Frequent
Rate of utilization
• Rate of digestion
• Rate of vitellogenin synthesis ?
• Rate of oocyte development
• Spacing of successive gonotrophic cycles
• Environmental influence
Frequencies of host visitation and feeding
Required quantity of blood not ingested in one feed
Required quantity of blood ingested in one feed
Physical or other restraints
• Defensive behaviour of the host
• Limitation in extensibility of abdomen

Figure 1. Role of nutrient intake on host visitation and feeding frequencies.

protease production is indicated in the work of Akov (1972), Briegel and Lea (1975), Fisk and Shambaugh (1952), Gooding (1973, 1974), Prasad (1979), Shambaugh (1954) and others. The active principle which induce protease activity appears to be in the serum (Gooding 1974; Langley 1966). *G. palpalis* requires serum albumin for normal digestion of ingested RBC and normal rate of fecundity (Takken 1980). Chapman (1985) has compiled results of various workers on factors of host's blood which induce protease activity in *Rhodnius*, *Aedes* and *Glossina*. Results have also suggested a control by MNC possibly through ecdysone (Briegel and Lea 1979). Secretogogue mechanism is now thought to be an immediate response to food consumption and control by hormones a later development (Applebaum 1985). Protein quality influences production of protease (Akov 1972; Gooding 1974). However, Yang and Davies (1968) showed trypsin activity to be almost identical in female *Simulium venustum* ingesting human, cow or duck blood or blood cells suspended in sucrose. There are reports for and against the possibility of source of blood meal influencing rate of digestion. Downe *et al* (1963) found that the source of blood had little effect on digestion rates of several spp. of *Aedes*, but did influence the digestion rate of *Mansonia perturbans*. Tempelis and Lofy (1963) found *Culex tarsalis* digesting blood from 3 different spp. of birds at different rates. No marked difference was noticed by Downe (1957) in the rate of digestion of horse and guinea-pig blood by several spp. of blackflies. Louse, *P. humanus* a highly host specific species showed difficulty in digesting guinea-pig blood. Weitz and Buxton (1953) found *C. lectularius* digesting human blood slowly. There is some objection that precipitin test used for the study of rate of digestion actually demonstrates the differential holding of blood fractions in the gut rather than rate of digestion.

It was already mentioned earlier that serum of vertebrate blood contain some substance which stimulate protease synthesis. Paradoxically enough, experiments have revealed that certain vertebrate blood sera also contain enzyme inhibitors.

Trypsin inhibitor is present in the sera of 17 vertebrates. Two of these have been purified and were shown to be associated with α-globulin fractions of the serum (Huang 1971a,b). An inhibitor associated with α-globulin of horse and human sera was shown to inhibit chymotrypsin from *Ae. aegypti* (Yang and Davies 1971). How the stimulatory and inhibitory factors present in the serum interact *in vivo* conditions and how these influence the rate of digestion of blood from different hosts needs clarification. For example chick blood serum contains more *Ae. aegypti* trypsin inhibitor than any other blood sample, yet chicks are good hosts for laboratory rearing of these mosquitoes. According to Gooding (1972) as digestion of only a small fraction of the ingested blood proceeds at a time, the chances of inhibitor being titrated out or destroyed would be more than if the enzyme were to mix with the whole blood meal at the same time. Whatever may this be, what biological function, if any at all, do these inhibitors play in the nutritional physiology of haematophagous insects is not clear. Judson (1986) mentions that inclusion of trypsin inhibitor in blood reduces or blocks the initiation of vitellogenesis.

Feeding and ovarian development inhibit host seeking behaviour (HSB) and subsequent feeding until after oviposition. Inhibition of HSB is related to the quantity of blood ingested. For effective inhibition the meal size should be more than 2·5 μl (Klowden and Lea 1979b). According to them this postprandial inhibition consists of two phases probably controlled by two different mechanisms. An immediate phase (distension inhibition) in *Ae. aegypti* is due to the distension of the anterior portion (1st 3 segments) of the abdomen. The second phase (oocyte inhibition) does not appear to be related to distension but due to humoral factor(s), as transfusion of haemolymph from gravid *Ae. aegypti* females 48 h post-blood-meal (PBM) into sugar-fed females inhibited host seeking behaviour (Klowden and Lea 1979a). These authors also state that it is unlikely that the increase in haemolymph amino acids/protein as a result of feeding (Armstrong 1968) is contributing to inhibition of host seeking behaviour, because saline enemas had the same effect as blood enemas. However, in rat fleas *X. cheopis* and *X. astia*, Geetha Devi and Prasad (1980) showed that injection of 50% bovine albumin into haemocoel reduced the quantity of blood ingested by unfed teneral females, while injection of a 10% albumin was sufficient to cause a quantitative reduction in blood intake by 7-day-old female fleas. This difference between unfed teneral and older females is attributed to the increase in total haemolymph protein in gonoactive females (Narayana Pillai and Prasad 1986). Surgical manipulations of ovaries have shown that these are not the source of the inhibitor in *Ae. aegypti* (Klowden and Lea 1979a). However, transplantation of fat body from blood-fed *Ae. aegypti* females into sugar-fed, inhibited the HSB indicating this as a source of the factor(s) (Davis and Bowen 1986). Beach (1979) showed that high titer of ecdysone inhibit feeding. If vitellogenins play any role in inhibition of HSB, it is not known. Inhibition of HSB starts about 30 h after blood meal, reaches a peak by 48 h and terminates after oviposition (Davis and Bowen 1986). Haemolymph titer of vitellogenin reaches a peak in *Ae. aegypti* around 27 h after the blood meal and remains in detectable level up to about 45 h (Ma 1986). Speaking of *Ae. taeniorhynchus* O'Meara and Evans (1977) state that as long as the ovaries remain undeveloped the females continue to be active blood feeders. Briegel and Lea (1979) showed that nonoogenic female *Ae. aegypti* secrete a quantum of nitrogen equivalent to total nitrogen normally observed in maturing ovaries. This shows that in nonoogenic females there is no protein build up necessary for vitellogenesis. If this has

any effect on feeding inhibition, does it have any role to play in rat fleas where female specific lipoglycoprotein fractions are detectable even in newly emerged unfed female fleas (Narayana Pillai and Prasad 1986)?. There appears to be a similarity between the condition of cecropia (Pan 1971) and rat fleas (Narayana Pillai and Prasad 1986) where vitellogenins appear in the haemolymph before vitellogenesis starts. Rapid diuresis soon after feeding concentrates the protein and a meal quantitatively adequate to initiate egg maturation is retained in the gut until shortly before oviposition. This retention may be due to cohesive nature of the meal, peritrophic membrane, physical barrier between mid and hind gut and/or hormonal feed back from the ovary. This retention at any rate ensures continued supply of nutrient for vitellogenin synthesis (Freyvogel and Staubli 1965; Nijhout and Carrow 1978; Rosenberg 1980).

Frequencies of host visitation and feeding would increase if adequate quantity of blood is not ingested. This may be due to the defensive behaviour of the host or physical restraints like limitations in the extensibility of the abdomen. There is considerable interspecific variation in defensive behaviour of hosts and the studies of Edman and his coworkers have shown these variations influence the success of feeding and quantity of blood ingested by mosquitoes (Edman and Kale 1971; Edman *et al* 1974; Webber and Edman 1972). Under such conditions if the mosquito has to lay eggs, repeated feedings would be necessary. Rat fleas *X. cheopis* and *X. astia* are typical examples where extensibility of the abdomen constraints ingestion of large quantity of blood required for the complete development of eggs. The small quantity of blood ingested along with low protein storage capacity of haemolymph make it necessary for these fleas to have frequent blood meals. In other words they have to live in close proximity of the host if they are to reproduce normally (Prasad 1986).

6. Influence of nutrition on male maturation

In general, spermatogenesis is completed by the time adult insect emerges. So it is unlikely that in most cases adult nutrition has any influence on spermatogenesis. However, in the testes of certain fleas, the exit of sperms into epididymis is blocked by a cellular plug which is dissolved only after feeding. Rothschild *et al* (1970) reported this to be the case in *X. cheopis*, *Nosopsyllus fasciatus* and *S. cuniculi.* They showed that male maturation (dissolution of the plug) is controlled by hormones of the host in *S. cuniculi.* Whereas in *X. cheopis* such a hormonal relationship is not seen. Blood feeding resulted in dissolution of the plug in 100% of *X. astia* and 75% of *X. cheopis* 24 h after the meal. When on a saline diet only 55% of *X. astia* and 40% of *X. cheopis* matured. These studies indicated a dependency of proteinaceous diet for male maturation. If those matured on a saline diet probably needed only a feeding stimulus and if this condition has any relation to larval diet is not known (Kamala Bai and Prasad 1979b). In the case of most ticks, feeding is intimately associated with male maturation. In most Metastriata, meiosis and spermatid production occurs only after an adult blood meal. Adult blood meal accelerates germinal development in males of *Argas* and *Ornithodoros* but not in *Otobius* and *Antricola.* As males of many *Ixodes* spp. do not feed, adult blood meal has no role to play in spermatogenesis (Oliver 1974). Many cyclorrhaphous Diptera (e.g. *S. calcitrans*, *H. irritans*) do

not become sexually active or become capable of inseminating females until after a proteinaceous meal. What exactly are the nutritional implications in these observations are not known (Anderson 1974).

7. Conclusions

Vertebrate blood as a diet has many fascinating facets. Arthropods have successfully adopted this as a source of nourishment probably even since Permian. Among insect orders Diptera, Siphonaptera, Anoplura and Hemiptera exhibit haematophagy. Feeding dichotomy both phytophagy and zoophagy is characteristic of nematoceran and brachyceran Diptera. But in these cases proteins derived from blood meal are essential for egg production, with exceptions of autogenous forms. These insects use a wide variety of hosts ranging from amphibians to mammals. Ticks are obligate blood feeders and blood is essential for egg production. Among mites members belonging to families Psoroptidae, Dermanyssidae and Macronyssidae exhibit haematophagy.

Both quantity and quality of the blood meal influence reproduction. Size of blood meal affect refeeding behaviour, midgut protease activity and of course fecundity. With certain exceptions, strict gonotrophic concordance is seen in many haematophagous insects where there is an alternation of blood feeding and oviposition. There is a positive correlation between quantity of blood ingested and number of eggs laid. Nutritionally (measured as rate of egg production) human blood appears to hold the least value compared to blood from other hosts for many blood sucking insects, but for *C. hemipterus*, blood from the reptile *Calotes versicolor* gave the least value. Nutritional inequalities can arise as a result of differences in the constituent amino acids. The nutritional value of any protein is a function of its amino acid composition.

Carbohydrates derived from nectar/plant juices enhance longevity and supplies energy for flight and does not have a direct role in egg production. But sugar diet in some cases inhibit or increase egg production. Rather poorly known are the roles of sterols, lipids, vitamins and salts in reproduction. An interesting example of sterol requirement is the case of rabbit flea *S. cuniculi* where the hormones of the host influence reproduction of the fleas. While sterols did not influence egg production of *Ae. aegypti*, deletion of cholesterol from the diet of rat fleas inhibited yolk deposition. Since lipids can be synthesized from carbohydrates and proteins, their absence from the diet does not adversely affect reproduction. However, arachidonic acid, a precursor of prostaglandin, is essential in the larval diet of *Cx. pipiens* for production of viable adults. Vertebrate blood being poor in vitamin B complex, the deficiency is made good by the symbiotic microorganisms. However, folic acid, thiamine and biotin are shown to influence egg production in certain species. But experiments on measuring the influence of vitamin B deficiency by feeding insects on deficient hosts should be viewed with caution as vitamin deficiency in the host can produce side effects which themselves could be injurious to the insects. Certain metallic ions like sodium, potassium, iron and calcium appear to influence reproduction of certain species.

Feeding in addition to supplying raw materials for egg production also induce the release of hormones from the endocrine glands. At least in some cases, the mecha-

nical stimuli from gut distension could trigger release of gonadotrophic hormones. It is possible that this could be achieved by chemical stimuli (peptides, amino acids?) from the diet.

Autogenous egg production is under the control of larval diet and genetic constitution.

Quantity of blood ingested and its rate of utilization decide the frequency of host visitation and feeding. Rate of digestion is one of the important aspects in utilization of the ingested food. Source of blood meal, rate of enzyme production, presence of enzyme inhibitors in the blood of hosts, environmental factors etc could affect the rate of digestion and so the rate of utilization. There is a definite postprandial inhibition of HSB at least in mosquitoes. The immediate cause may be abdominal distension and later inhibition is thought to be mediated by humoral factor(s). Ecdysone is known to inhibit feeding. If vitellogenin in the haemolymph has any influence, it is not proved experimentally.

Adult nutrition has no direct influence on spermatogenesis in insects, male maturation is influenced by hormones of the host in rabbit fleas and feeding on a proteinaceous diet (nutritional?) in rat fleas. Cyclorrhaphous Diptera become sexually competent only after a blood meal. Adult blood meal has a decisive role to play in spermatogenesis and maturation of many species of ticks.

A scan through the literature cited in the present review would show that a large quantum of work on nutrition and reproduction has turned out centering around mosquitoes. Much more work is needed to understand the mechanisms involved in other groups like fleas, bugs and ticks.

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Feeding and reproduction in vector mosquitoes

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Abstract. Mosquitoes depend on high fecundity to compensate for heavy immature mortality. Important factors affecting fecundity are (1) Body size, dependant on larval nutrition (2) Amount of blood ingested (3) type of blood ingested. With the development of highly sensitive techniques for determination of bloodmeals of wild mosquitoes many puzzling variations in host selection behaviour are now capable of explanation.

Keywords. Mosquitoes; fecundity; bloodmeal identification.

1. Introduction

The mosquitoes are r-strategists, falling within Odum's definition of species which display a high ratio of reproductive to maintenance effort (Odum 1983). How successful this strategy is for species survival is borne out by the fact that strenuous human efforts have totally failed to eradicate even a single species of mosquito from any part of the world, except in the case of *Anopheles gambiae* from Brazil and Egypt, where the species was a recent entrant and had not established itself. Mosquitoes depend on a high fecundity to compensate for heavy immature mortality. Mean mortalities of 99·1 and 97·6% respectively were recorded for *Culex vishnui* and *A. peditaeniatus* larvae in Philippine paddy fields, of which about 90% could be shown to be due to predators, including Anisoptera (Mogi *et al* 1984). Predation was not important in *C. quinquefasciatus* breeding in wells in Delhi villages, where mortality was strongly density dependant (Rajagopalan *et al* 1976). In both cases the key to survival was high fecundity, which made it possible for the species to tide over adverse periods and to build up high population levels in favourable seasons, in spite of the operation of strong mortality factors. As in other insects, fecundity in mosquitoes is closely associated with nutrition both at the adult as well as at the larval stage. These relationships have been extensively studied, particularly since their blood-feeding habit is linked with the transmission of pathogens of medical importance.

2. Fecundity

The average number of eggs laid during one gonotrophic cycle is a species characteristic, and varies considerably between species. In general the larger species lay more eggs than the smaller species. Even within the same species, individual egg batches show considerable variation. Factors affecting fecundity can be grouped under 3 heads; (i) body size, (ii) amount of blood ingested and (iii) type of blood ingested.

2.1 *Body size*

The number of eggs laid shows a positive correlation with the size of the female in several genera of mosquitoes. In *Aedes aegypti* the total number of ovarioles available for development in both the ovaries showed a positive correlation with the size of the female, varying from 50–150 (Colless and Chellapah 1960). In addition, large females of this species ingested more than twice as much blood as small females (Bar-Zeev 1957). Undersized females of *A. aegypti* generally need 2 blood meals before they can develop eggs (Macdonald 1956). Body size itself is determined by nutritional status, large females being produced from well fed larvae, and undersized adults under conditions of starvation and overcrowding. This provides a neat feed-back mechanism for reducing egg production when food resources are a limiting factor.

Larval nutrition plays an important role in the phenomenon of autogeny, which is recorded in several genera, but has been best studied in *C. pipiens molestus*. Autogenous mosquitoes are able to lay a single egg raft without feeding, but require a blood meal for maturing subsequent egg batches. Autogenous females use reserves carried over from the larval stage to develop the first batch of eggs. Some workers have found that poor larval nutrition reduces the number of eggs laid by autogenous females, but that even larvae near the starvation limit produced females which were all autogenous. Other workers found that poor larval nutrition lowered both the percentage of laying females as well as the number of eggs produced by an autogenous population (Clements, 1963).

2.2 *Amount of blood ingested*

In autogenous mosquitoes engorging by the female stimulates the secretion of a gonadotrophic hormone, resulting in the development of the ovaries. The hormone thus links ovary development with the supply of nutrients. It has been shown experimentally that distention of the abdomen for a sufficient period, even with liquids other than blood, stimulates afferent nervous impulses leading to the release of gonadotrophic hormones by the neurosecretory cells of the brain and the corpora allata (Larsen and Bodenstein 1959). The subsequent development of individual oocytes depends on the availability of nutrients. After a sufficiently large blood-meal the majority of the follicles in the resting stage begin to develop, but a number of them degenerate during the early stages of oocyte development. The number of follicles degenerating is greater after a nutritionally inadequate meal (Hosoi 1954) and it is also high in older females which have passed through several gonotrophic cycles (Detinova 1949).

Competition between follicles for available nutrients has been demonstrated by transplanting ovaries at the resting stage into the abdomens of females of the autogenous females of *C. pipiens molestus*. When the host was less than 16 h old the transplanted ovaries developed to maturity and suppressed development of the host ovaries. When the host was however, 50–65 h old, its own ovaries continued to develop, but the transplanted ovaries did not (Larsen and Bodenstein 1959). The exact nature of the inhibition was not clear.

2.3 *Type of blood ingested*

Early work in which blood fractions were fed to mosquitoes suggested that protein

was the only essential requirement for egg production, and this was confirmed when synthetic diets of amino acids were fed to female *A. aegypti* (Dimond *et al* 1956). Eight amino acids were essential for complete development. These were arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan and valine. In addition, when either histidine or methionine was omitted from the diet, a few eggs were laid a few days after the start of the experiment, but not later. This was interpreted to mean that these two amino acids were carried over from the pupa in small quantities, but when these stores were depleted no further egg production occurred. Ten amino acids were therefore essential. Addition of cystine and glutamic acid to the diet enhanced egg output.

It is generally believed that mosquitoes will develop more eggs when fed on the preferred natural host. However, little work has been done on this aspect. *C. pipiens* laid on an average 40 eggs/mg of human blood and 82 eggs/mg of bird blood (Woke 1937). It is possible that the nucleated erythrocytes of bird blood are more nutritive than the anucleated erythrocytes of mammals. However, addition of nucleic acids to the diet of *A. aegypti* did not raise egg production. In the same species, egg production was lower when females were fed on whole blood of man, sheep or ox, in which the isoleucine content of the haemoglobin is low, as compared with the high egg production when fed on whole blood of pig or rabbit, which have a higher isoleucine content. Addition of isoleucine to human, sheep or ox-blood raised the level of egg production to that obtained with pig or rabbit blood (Lea *et al* 1956).

3. Feeding preferences of wild mosquitoes

Host selection by adult mosquitoes in nature will, therefore, play a role in population dynamics by influencing fecundity. It has also a direct bearing on vectorial capacity, species showing a higher degree of anthropophily being more efficient in transmitting pathogens to man. Much attention, has therefore been devoted to the determination of the source of blood meal of wild mosquitoes. Basically the test consists of antigen-antibody reactions between serum antigens of mosquito blood-meals of unknown origin and specific antisera, resulting in visible precipitates. Specific antisera of representing animals of the area can be raised in cockerels and rabbits. The test can be conducted either in solution in small glass tubes (precipitin test) or in semi-solid agarose medium (agarose gel diffusion technique and radial immunoassay). The speed and sensitivity of the agarose gel assay can be enhanced by electrophoretic methods. In the precipitin test the antigen-antibody reaction is seen as a ring; in agar gel diffusion and electrophoretic methods as bands/arcs; and in radial immunoassay as circular zones. Of the 4, the electrophoretic method is the most sensitive, followed by gel diffusion, radial immunoassay and precipitin test (Bheema Rao 1984).

For most purposes it is sufficient to have relatively broadly reacting antisera, and to distinguish between bloods of humans, birds, bovines and so forth. However, it is possible to raise antisera which are order specific and even species specific (Tempelis and Reeves 1962a, b), and this has been used in the study of vectors of viral encephalitides, where particular orders and families of birds are known to be amplifying hosts of viruses. Thus Reeves *et al* (1963) studied the feeding habits of *C. tarsalis*, the vector of Western equine and St. Louis encephalitis, in California. 3,310 engorged females were tested. In summer 84·4% of the females had fed on birds, which included chickens, doves and passerines. Cattle, dogs, horses, rabbits and cats

accounted for 13·9% of the feeds. During winter, 98% of the feeds were on birds, mostly passerines, while only 1·4% were on mammals. This reflects the flocking together of passerines during winter, and the absence of doves and chickens. It is also technically feasible to distinguish different haemoglobin types of mosquito blood-meals of human origin. Thus, by testing blood-fed *A. gambiae* and *C. quinquefasciatus* collected inside bed-nets under which mother and baby pairs with different haptoglobin types slept, it was possible to show that the mothers were fed on much more by mosquitoes than the babies (Boreham *et al* 1978).

The choice of host in nature depends on (i) host availability as well as (ii) innate species preference. The influence of host availability is clearly demonstrated in the results of precipitin tests carried out on blood meals of *C. quinquefasciatus* collected in villages near Delhi. Among specimens collected in the centre of the village 93·3% was fed on man and 1·7% on birds. At the periphery 64·2 and 25·2% were fed on man and bird respectively, while among those mosquitoes collected in pump houses outside the village only 17·7% were fed on man while 53·0% were fed on bird. During the hot season, when these collections were made, heavy mosquito breeding was found in agricultural wells, which were not in use at this season. Mynas and pigeons were found breeding in the Persian wheel containers and mud embankments, and presumably the mosquitoes were able to maintain a cycle independent of the blood sources within the village (unpublished data of the ICMR-WHO Research Project on Genetic Control in Mosquitoes, New Delhi). Seasonal shifts in choice of hosts, as in the case of *C. tarsalis* in California cited above, are often due to seasonal shifts in host availability; in this case due to migration of some species of birds out of the area and tethering of domestic stock indoors during the winter. *A. culicifacies*, a normally zoophilic species with a strong preference for bovine blood, shows seasonal changes in feeding behaviour in Pakistan, where there is a higher proportion of manfeeding in the summer months when villagers sleep out of doors, as compared with the winter months when they sleep indoors under quilts (Reisen and Boreham 1979)—a different kind of host non-availability. This opportunistic host selection behaviour of mosquitoes, particularly those species which are basically zoophilic, can be exploited to divert them from human hosts (zooprophylaxis). It was shown that keeping cattle under the same roof as man (mixed dwelling) increased contact between *A. aconitus* and man, while if the cattle were kept in open cattle sheds, without roof or walls, away from human dwellings, man-vector contact fell to extremely low levels, as shown by the human blood index as well as direct collections from human bait (Kirnowordoyo and Supalin 1986).

While host availability can alter mosquito feeding behaviour according to circumstances, there are nevertheless strong innate preferences for particular hosts, which are species specific. Mosquitoes may be broadly classified as anthropophilic or zoophilic according to their preference for human blood or animal blood respectively. Some of the most dangerous disease vectors are anthropophilic, for example *A. gambiae*, *A. minimus* and *A. fluviatilis*, vectors of malaria, *A. aegypti*, the vector of dengue haemorrhagic fever and *C. quinquefasciatus*, the vector of bancroftian filariasis. There are also efficient zoophilic vectors of malaria, for example, *A. culicifacies*, and zoonotic diseases of man are primarily transmitted by zoophilic mosquitoes, for example *C. tritaeniorhynchus* and allied species which transmit Japanese encephalitis virus. The feeding preferences of these species were extensively investigated in North Arcot District and clear differences in the feeding behaviour of

various common species of mosquitoes emerged. In the same villages, with the identical hosts available to them, species of the *C. vishnui* subgroup, all important vectors of JEV, were strongly attracted to cattle, and there were few man feedings reported. On the other hand *C. bitaeniorhynchus* showed a higher percentage of feedings on birds than on cattle, and 4·6% were fed on man (Christopher and Reuben 1971).

Blood-meal testing has been carried out most extensively on species of *Anopheles*, particularly in the early years of the National Malaria Control and Eradication Programmes. A number of puzzling anomalies were revealed of the same species exhibiting anthropophilic behaviour in one geographical area and zoophilic behaviour in another. These differences could not always be related to host availability. It has now been shown that many taxa, previously considered to be species, are in fact complexes of sibling species which are morphologically identical, but can be differentiated by cytological or electrophoretic analysis. *A. culicifacies* is in reality a complex of 4 sibling species provisionally named A, B, C and D. In the Thenpennai riverine tract, 434 specimens of species A were tested, of which 21·4% had fed on man, while on 1·8% of 398 tests on species B were positive for human blood. Since species A predominates in the dry season, and species B in the wet season, the population of *A. culicifacies* shows an apparent seasonal change in feeding behaviour which is due to the change in proportion of the different component sibling species (Tewari *et al* 1984). Research in this area is rapidly advancing and many anomalies in host selection patterns will soon be capable of explanation.

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Host preferences in some pentatomids and related impact on the fecundity of their parasitoids

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Abstract. Two species of polyphagous parasitoids, *Gryon* sp and *Telenomus lucellus*, parasitising the pentatomids *Acrosternum graminea* and *Agonoscelis nubila* were subjected to host preference studies and consequent impact on the rates of parasitism of these host eggs. Chemical composition of these eggs were correlated with the age specific fecundity of the parasitoids. Polyphagous pentatomids like *Acrosternum graminae* had varying fecundity on different host plants like *Cleome viscosa*, *Gynandropsis pentaphylla*, *Leucas aspera*, *Croton sparciflorus*, *Acalypha indica* etc and this variation was found to depend on the chemical parameters of these host plants. The overall egg output of the pentatomids was also found to influence the percentage of parasitism.

Keywords. Parasitoids; pentatomids; tritrophic interactions; life table studies; intrinsic rate of natural increase; fecundity index; biochemical analysis.

1. Introduction

The conceptual and factual basis for understanding the multifaceted nature of trophic interactions between plants, insects and parasitoids/predators has been established very clearly in the long progression of experiments that has led to our modern understanding of Batesian-Mullerian mimicry complexes (Rothschild 1972; Gilbert 1983; Duffey *et al* 1986). A more dramatic, but seemingly unnatural example of the movement of chemicals through multiple trophic levels was demonstrated by showing the ability of insecticides to bioaccumulate at given foci in the food chain (Kilgore and Li 1976). The nutritional suitability of a host was proposed by Flanders (1937) and Salt (1938) to be important for the survival of a parasitoid. House (1961) concluded that the nutritional needs of parasitoids are probably similar to most other organisms, and the problem of nutritional suitability may not be due to lack of specific nutrients or accessory growth factors in the host, but the quality and ability to obtain certain nutrients present in the host tissues at the proper time and to compete with the host tissues for the available nutrients. Yet another important factor is the amount of nutrients available to the parasitoid since a host (egg) is a finite food resource which because of its size, age and nutritional history may affect parasitoid development and reproduction (Vinson and Iwantsch 1980). Smaller host eggs yield smaller parasitoids with reduced emergence, fecundity and longevity or more males (Vinson and Iwantsch 1980). Female parasitoids may have the ability to assess host size and deposit fertilised or unfertilised (male) eggs accordingly (Nazato 1969), or they may deposit eggs of both sexes with preferential survival of one sex in larger hosts (Holdaway and Smith 1932). Within solitary endoparasitoids, Miles and King (1975) reported decreases in the parasitoids developmental time with increasing host age at the time of parasitoidism, which may be due to nutrients in older hosts being more abundant (Vinson 1984). The present paper attempts to highlight the tritrophic level of interactions between the host plant, the pentatomid host and its

natural enemies especially with reference to the flow of lipids through the system in greater detail.

2. Materials and method

2.1 *Biochemical analysis*

The chemical composition of the various host plants and the eggs of pentatomids were analysed using the following standard procedures. Total carbohydrates (Dubois *et al* 1956); total phenols (Bray and Thrope 1954); total proteins (Lowry *et al* 1951) and total lipids (Folch *et al* 1957).

In order to analyse the qualitative fatty acid profile of the host plants and the eggs of the host, the total lipids were extracted using the following procedure—to the material was added 10 ml of ethanol, 3 ml of 28% ammonium hydroxide, 25 ml of petroleum ether and 25 ml of diethyl ether in a separating funnel, this mixture was shaken for 5 min and allowed to stand for separation for 20 min. The bottom layer was drained off and the ether fraction was dried to which 3 ml of 0·5 N NaOH in methanol was added and heated in a steam bath for 15 min. To this 5 ml of distilled water was added and 2 N HCl were added slowly until the pH reached approximately 2. The fatty acid methyl esters were then extracted into 5 ml of petroleum ether and 5 ml of diethyl ether from the acidified methylated lipid extract. Fatty acids were analysed by the Hewlett Packard HPLC system at 230 nm using an Hypersil ODS 5 μm column with water and acetonitrile as solvents at a flow rate of 0·45 ml according to the gradient programme as per Schuster (1985). The retention time and area percentage of fatty acid methyl esters were recorded.

2.2 *Life table*

Five adult female parasitoids of both species (*Gryon* sp and *Telenomus lucellus*) soon after emergence were allowed to mate with their male counterpart *ad lib* and these pairs were isolated and kept in separate plastic vials along with 25 host eggs. They were also provided with a cotton bud soaked in dilute honey. Every day these females were provided with 25 fresh host eggs and the previous egg masses were labelled and kept for observation of the emergence of the parasitoids. The use of low parasitoid/host ratio resulted in high rates of parasitism. Thus the number of eggs laid by the parasitoids were estimated by summing up the following values—the number of emerging parasitoids and also their sex ratio. These observations were made till the death of the female parasitoid. In the case of the death of the male partner it was substituted by another male.

The life tables were constructed with the help of fecundity and later the intrinsic rates of increase of population of parasitoids were calculated using Birch's (1948) formula as elaborated by Watson (1964).

$$e^{-r}\, m^X\, l_x m_x = 1,$$

where e is the base of natural logarithms, X is the age of the individuals in days, l_x is the number of individuals alive at age X in proportion of one and m_x is the number of female offspring produced per female in the interval x while R_o is the sum of the

products of $l_x m_x$ the rate of multiplication of population for each generation measured as the female offspring produced. The approximate value of cohort generation time T_c was calculated as follows

$$T_c = \frac{l_x m_x X}{l_x m_x}.$$

The arbitrary value of innate capacity for increase r_c was calculated from the formula

$$r_c = \frac{\log R_o}{T_c}.$$

This was an arbitrary value for r_m and value of r_m upto two decimal places was substituted in the formula until the two values of the equation were found immediately above or below 1096·6. The two values of $\Sigma e^{-r} m^X l_x m_x = 1$ were plotted on the horizontal axis against their respective arbitrary r_m value on the vertical axis. The two points were joined to give a line which intersected a vertical line drawn from the desired value of

$$e^{-7} r_m \times l_x m_x \ (1096{\cdot}6).$$

The point of intersection gave the value of r_m accurate to 3 decimal places. The precise generation time T was then calculated from the formula:

$$T = \frac{\log R_o}{r_m}.$$

The finite rate of increase (λ) was calculated as $e^{-r} m$.

2.3 *Fecundity studies*

To study the fecundity of the pentatomids, they were reared on their respective host plants i.e. *Acrosternum graminea* (Fabricius) on *Cleome viscosa* L, *Gynandropsis pentaphylla* DC, *Acalypha indica* L, *Croton sparciflorus* Mor. and *Leucas aspera* Spr; while *Agnoscelis nubila* (Fabricius) being monophagous was reared on *Leucas aspera*. Fecundity of the parasitoids were analysed by providing 25 eggs per day for 3 days after which the parasitoids failed to lay any eggs.

3. Observations

The results showed that the longevity of ovipositing *Gryon* females ranged from 5–18 days (average 12·5). The number of progeny produced ranged from 46–78 (average 58). The male : female ratio of progeny ranged from 1 : 6 (average 4·6). The first adult mortality within the cohort occurred on the fifth day after oviposition and mortality increased thereafter (figure 1). The duration of the immature stages lasted for 8 days. The adults attained maximum mean progeny production per day (m_x) of 6·250 female per female on the third day and production ceased by the twelfth day after oviposition (figure 1). The innate capacity for increase was 0·2810 (figure 2) per female

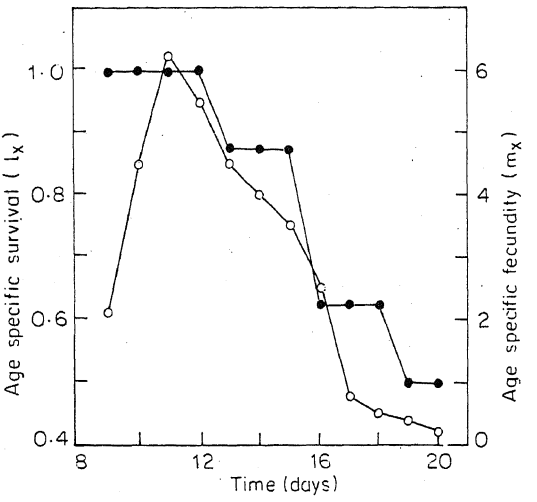

Figure 1. Daily age specific survival and age specific fecundity of *Gryon* sp.

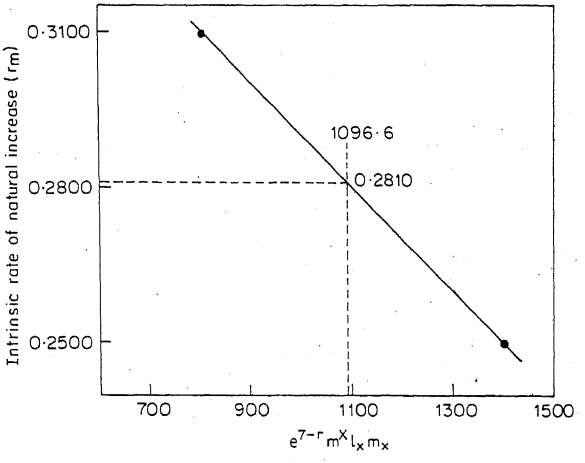

Figure 2. Determination of the intrinsic rate of increase (r_m) of *Gryon* sp.

per day and the population of this parasitoid would tend to multiply by 31·5581 in a generation time of 12·2841 days in the present conditions. The finite rate of increase was 1·324 during the reproductive period of 12 days.

In contrast to the above analysis the life table analysis of *T. lucellus* on the same eggs of *Acrosternum graminea* showed that the longevity of the ovipositing female ranged from 3–15 days and the number of progeny produced ranged from 30–64 (average 36·8). The sex ratio was 1 male:9 females (average 6·9) and the first adult mortality within the cohort occurred on the third day after the commencement of oviposition and the length of time spent in immature stages was 7 days. The adults attained maximum mean progeny production per day (m_x) of 9·66 females per female

on the fourth day (figure 3) and production ceased by the tenth day and the overall innate capacity for natural increase (r_m) was found to be 0·3640 per female per day (figure 4). This study was mainly done to calculate the intrinsic rate for natural increase because it has been used as a bioclimatic index for rating the efficiency of these parasitoids. It was also observed that the population of *T. lucellus* (Nixon)

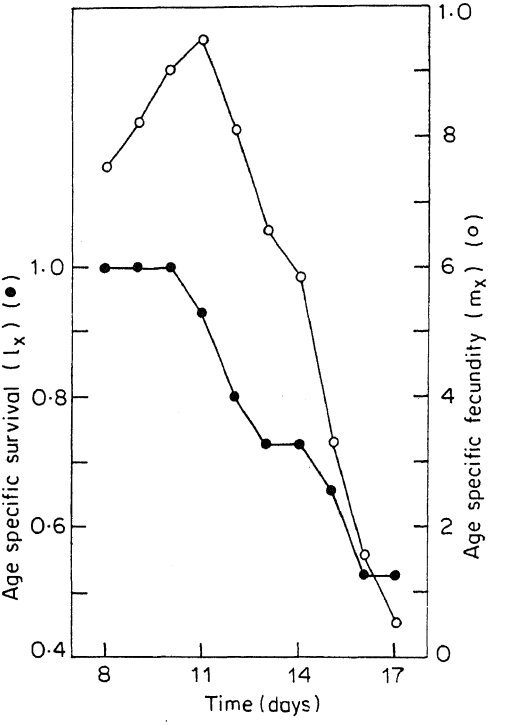

Figure 3. Daily age specific survival and age specific fecundity of *T. lucellus* (Nixon).

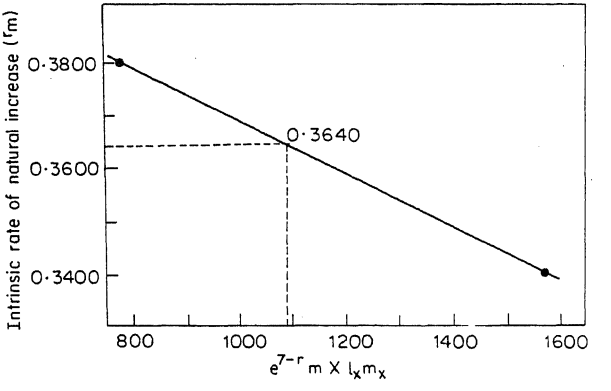

Figure 4. Determination of the intrinsic rate of increase (r_m) of *T. lucellus*.

would tend to multiply 53·231 in a generation time of 10·919 days in the present conditions. The finite rate of increase was 1·440.

Further observations revealed that though the r_m value was more for *T. lucellus*, *Gryon* sp was more efficient in the rates of parasitisation because of a radical difference in the general behaviour. It was found that *Gryon* could tolerate other individuals of the same species, parasitising simultaneously on the same egg mass, while a female *T. lucellus* would not tolerate another individual within the vicinity of the egg mass. Scanning electron microscopic studies also highlighted the presence of some receptors on the ovipositors of these parasitoids which in turn would aid in an analysis of the egg content, prior to feeding. It was also observed that these parasitoids spent the initial time on the act of host feeding which was presumed to supplyment its natural diet (honey dew).

The chemical composition of the host plants (table 1) revealed that an optimum proportion of all the dietary constituents is a basic requisite for the fecundity of the pentatomids. The data presented in the table clearly shows that high carbohydrates, proteins, phenols and lipids as found in *C. viscosa* results in the maximum egg production of *A. graminea* (126 ± 8·75) (table 2). In *A. indica* there was a higher percentage of carbohydrates and protein than in *C. viscosa* and almost equal quantity of phenols, but the fecundity on this host was comparatively less (102 ± 4·9) (table 2) and this can be attributed to the low dosage of lipids present within *A. indica*. Egg laying was the least in *C. sparciflorus* (46 ± 5·9) (table 2) though it had a high level of carbohydrates, but low amount of other components. Lower fecundity in *L. aspera* could be due to the low amount of proteins and lipids, while in *G. pentaphylla*, it could be due to the least amount of carbohydrates, low proteins and phenols though it had a higher amount of lipids. On the other hand the fecundity of *A. nubila* was 140 ± 10·8 on its host *L. aspera* and this pentatomid was found to be monophagous. The fecundity index and the average number of eggs per oviposition (table 2) of *A. graminea* also shows a higher value on *C. viscosa* over all the other host plants.

Table 1. Chemical composition of the host plants (mg/g).

Host plants	Family	Carbohydrates	Proteins	Lipids	Phenols
C. viscosa	Capparidaceae	125	62	22	28
G. pentaphylla	Capparidaceae	55	50	21	12
A. indica	Euphorbiaceae	190	65	10	27
C. sparciflora	Euphorbiaceae	150	39	11	19
L. aspera	Labiatae	160	51	15	30

Table 2. Fecundity of pentatomids *A. graminae*.

Host plants	Fecundity	Fecundity index	Oviposition	Average number of eggs per oviposition
C. viscosa	126 ± 8·75	21	4–7	25·2
A. indica	102 ± 4·99	17	4–8	19·5
G. pentaphylla	81 ± 11·60	13·6	6–12	9·09
C. sparciflorus	46 ± 5·9	7·8	6–9	5·11
L. aspera	67 ± 10·8	11·16	5–8	8·0
A. nubila				
L. aspera	140 ± 10·8	11·16	3–5	8·0

Experiments on the fecundity of the parasitoids in order to analyse the importance of supplementary feeding clearly shows that both the egg parasitoids i.e. *Gryon* sp and *T. lucellus* preferred the eggs of *A. graminea* over *A. nubila*, though in texture the chorion of the former was thicker than the latter. Hence this enhanced fecundity of both the parasitoids (table 3) within the eggs of *A. graminea* and this could be due to some dietary constituent derived from the egg at the time of host feeding.

Figures 5 and 6 show the results of the HPLC analysis of fatty acid methyl esters and it clearly depicts the presence of stearic acid in all the host plants as well as the chemical content of the egg. Based on the retention time it was also observed that the unsaturated fatty acids which got separated within the column before 5 min also formed a high percentage within the plants (except *C. sparciflorus*) and eggs. The low

Table 3. Fecundity of the parasitoids.

	Eggs	
	A. graminae	*A. nubila*
Gryon sp	20±3·16	16±1·5
	12±3·16	7±2
	6±2·34	2±1·41
T. lucellus	18±2·14	14±2·34
	8±1·48	6±1·62
	4±1·21	2±1·48

Fresh eggs given at 25 eggs per day for 3 days.

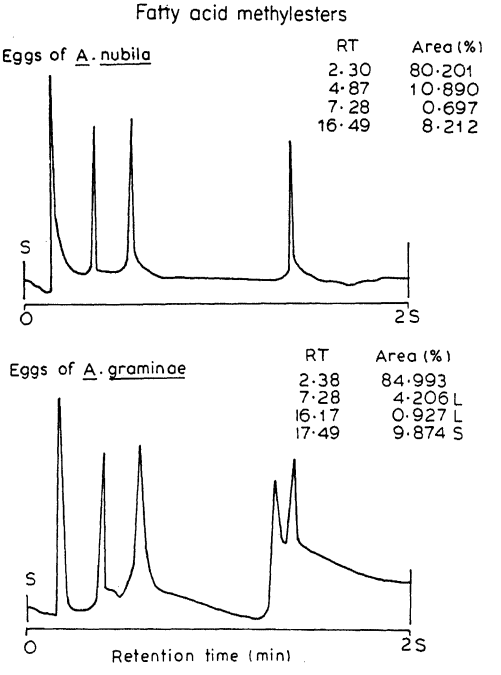

Figure 5. HPLC analysis of fatty acid methyl esters of eggs of *A. graminea* and *A. nubila*.

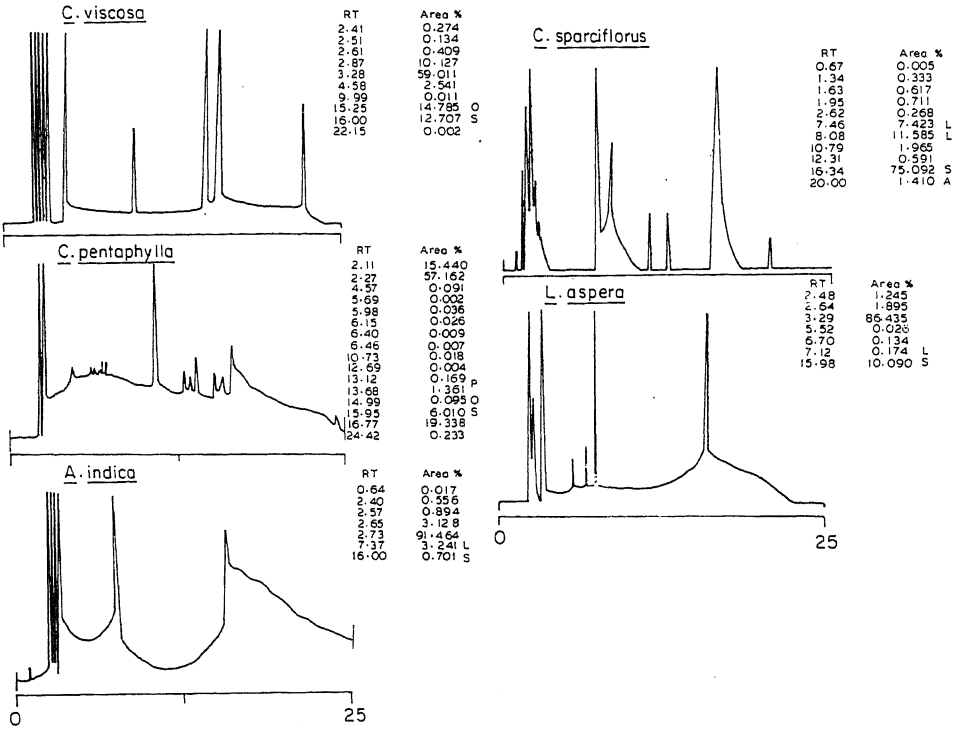

Figure 6. HPLC analysis of fatty acid methyl esters of host plants.

concentration of these fatty acids within *C. sparciflorus* could be the cause of low fecundity of *A. graminea* on this host plant.

4. Discussion

The development of general theories on plant defensive strategies against insect herbivores has received considerable attention because the plant/herbivorous insect relationship has provided a moderately tractable experimental system with which to investigate the role of biotic factors as a potentially main determinant in regulating animal abundance. Of particular importance is the role of plant quality (i.e. nutritional value, texture and architecture and defensive characteristics) in determining the population dynamics of herbivorous insects and their coevolution with plants (Atsatt and O'Dowd 1976; Denno and McClure 1983; Kareiva 1983; Price 1983; Rhoades 1983; White 1978). Price (1983), Whitham (1983) and McClure (1983) indicated that an assessment of plant fitness as well as insect fitness must be integrated into the nature of tritrophic interactions. The macroscopic architecture of individual plants or communities of plants are known to influence the ability of parasitoids to locate hosts (Hassell and Waage 1984; Kareiva 1983; Price 1983; Price *et al* 1980; Vinson 1976). At a more microscopic level, physical factors such as leaf-shape, leaf colour, the presence of trichomes etc also modify the ability of parasitoids to locate and utilise hosts (Price 1983; Price *et al* 1980; Vinson 1976). Integrated with the above

modifiers is the ability of plant odours (damaged and undamaged states), host-insect produced kairomones and/or host-insect behaviour/physiology conditioned by established parasitoids or host-plant geno/phenotype to modify the ability of parasitoids to effectively locate and utilise hosts (Duffey *et al* 1986; Beckage and Riddiford 1983; Fritz 1982; Greany *et al* 1977; Hagstrum and Smittle 1977; Holmes and Bethel 1972; Levin *et al* 1982; Lewis *et al* 1977; Loke *et al* 1983; Nordlund *et al* 1977; Prokopy and Webster 1978; Roth *et al* 1982; Pair *et al* 1983). Finally at the microscopic chemical level is the ability of plant natural products, after ingestion by the host insect, to modify the efficacy of parasitoids.

This paper has thus highlighted the passage of fatty acids through 3 tropic levels and also shows that the host feeding behaviour of the parasitoids could enhance its fecundity. Insects are well known to accumulate fatty acids in high concentrations, especially at physiological stages of development preceding periods of non feeding or in maturing females for deposition in eggs. Certainly the quality of these host's food has a great bearing on the growth, development and survival of parasitoids because it is thought that the food modifies the quality of the host for a given parasitoid (Cheng 1970; Shapiro 1956; Smith 1957; Zohdy 1976). At present, the paucity of work in these directions has resulted in the lack of knowledge regarding these complicated mutual interactions which could give an idea of a comprehensive physiological mechanistic theory of tritrophic interactions. Studies on the role of host nutrition as in *A. graminea* has indicated that the addition/subtraction of specific nutrient could alter the reproductive capacity of the host insect. Further studies on other dietary components could throw more light on these interactions.

Acknowledgements

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Impact of predation and food utilization on reproduction of *Diplonychus indicus* and *Ranatra filiformis*

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Abstract. Impact of predation and food utilization on reproduction of the water bugs — *Diplonychus indicus* and *Ranatra filiformis* was studied. In *Diplonychus indicus*, the food quality affects the rate of predation as well as food utilization. Allometric growth of various body parts and the longevity of each nymphal instar of the bug showed distinct variation, when exposed to individual prey item viz *Culex*, *Anopheles* and *Aedes* larvae and fish fingerlings. In *Ranatra filiformis*, a distinct pattern of oviposition in relation to the increased rate of predation on *Culex* larvae was observed. The significance of such variations in reproductive activities of these water bugs was discussed.

Keywords. Allometric growth; longevity; energetics; oviposition.

1. Introduction

Nutritional ecology is central to proper interpretations of manner of feeding, reproduction, defense, habitat selection and such other life history phenomena in arthropods (Scriber and Slansky 1981). With regard to factors that promote oviposition, embryonic development, growth and sexual maturity, starvation or nutritional deficiency is felt to be a major cause. Davis (1964) has reported that resorption of oocytes in hemipterous insects is governed and promoted by the nutritional status even in mated females. When food quality is changed, the subsequent rate of development, body composition and growth of arthropods differed significantly (Waldbauer 1968; Mueller *et al* 1973; Blumberg and Swirski 1974). To predators, the mobility is vital among properties of the prey item. In aquatic hemipterous bugs, the predation is categorised as chase and capture the prey (Peckarsky 1982), sit and wait in ambush (Hoffmann 1927) and resting-questing attitude (Menke 1979).

If nitrogen proved to be a limiting nutrient for the growth as suggested in *Pieris rapae* (Slansky and Feeny 1977), then the first objective of the present study was to verify if growth during postembryonic development is related more closely to the nutritional status and to the energetics of an active predator—*Diplonychus indicus*. The second objective was to study the interaction between the sex of the bug and the efficiency of utilization of the food provided.

The third objective was to examine the impact of specific diet on life fecundity of a matured female and ovipositional strategy in *Ranatra filiformis*.

2. Materials and methods

2.1 *Longevity and allometric growth*

To record the impact of nutrition on longevity and allometric growth on *D. indicus*, the encumbered males were maintained in the laboratory. First nymphal instar that

emerged and its successive stages of development till adulthood were fed with *Culex*, *Anopheles* or *Aedes* larvae with fish fingerlings as control. The life span of each nymphal instar with specific diet was recorded. Various body measurements of all nymphal instars fed with specific diets were measured. Data obtained were fitted to Huxley's (1924) exponential formula $Y=bX^k$, where Y is the allometrically growing organ, b the growth index, X the body length taken as reference measurement and k the equilibrium constant by which Y grows in relation to X throughout the ontogenetic stages and the significance of growth was derived statistically.

2.2 *Energetics of nymphal instars*

All nymphal instars of *D. indicus* were starved for 24 h prior to experiments. Their predatory efficiencies were tested against 4th age group of *Culex* and *Aedes* larvae at different densities. To assess the food utilization, the scheme of energy balance of Petrusewicz and Macfadyen (1970) namely $C=P+R+F+U$ was followed, wherein C, food consumed; P, growth attained; R, respiration; F, faeces and U, nitrogenous wastes. From the data obtained, the assimilation and the conversion efficiencies, rates of consumption, assimilation and conversion were calculated for all developmental stages. Tissue samples of these experimental ones were dried at 105°C. The calorific value of each sample was determined by incinerating them in a semi micro oxygen bomb calorimeter following the procedure of Pandian and Madhavan (1974).

2.3 *Food utilization in adults*

Freshly moulted males and females of *D. indicus* were maintained in the laboratory with 4th age group of *Culex* larvae. After feeding them for 20 days, the experiment was suspended and the bugs were treated to estimate the assimilation and conversion efficiencies, rate of feeding, assimilation and conversion as cited earlier.

2.4 *Statistical analysis*

Data obtained were subjected to statistical analysis of variance to derive the significance of differences in the above experiments.

2.5 *Life fecundity and oviposition*

Fifth nymphal instar of *R. filiformis* were collected and maintained in the laboratory with 4th age group of *Culex* larvae as the specific diet at 28 ± 1°C with a daily regime of 12 h light and 12 h darkness.

Males and females obtained soonafter moulting from 5th nymphal instar were kept in pairs and were fed normally with *Culex* larvae. Stems of *Hydrilla* were provided as ovipositional cites in order to record the percentage of ovipositive females, rate of reproduction, total number of eggs produced during its life time, distribution of eggs on the floating vegetation, adult longevity and the percentage of hatching success. Number of eggs per batch of a female served as the index of reproductive potential. First nymphal instar that emerged and the successive stages it moulted with *Culex* larvae as diet were also recorded.

3. Results

3.1 *Nutrition and life span*

Table 1 shows that the duration of post embryonic stages of *D. indicus* in the laboratory reared population simulating natural conditions and providing food *ad libitum* varies from 80–90 days. The longevity of I nymphal instar and II were 8·7 days, III for 10·6 days, IV for 24·5 days and V for 37·5 days. However, when they were fed with a specific test food, the longevity of each nymphal instar varied significantly. On comparison, the duration of *Culex* fed ones was higher throughout the post embryonic development than those fed with other test food. Duration of development of all nymphal instars was found to be very low when fed with fish fingerlings.

3.2 *Allometric growth in D. indicus*

Figure 1 shows the measurement of the total body length of all nymphal instars plotted against time in days of the predator stages. Second and third instars of *D. indicus* showed maximum body length when fed with Culicine larvae. The fourth nymphal instar showed maximum body length when fed with anopheline larvae. The fifth nymphal instar showed maximum body length when fed with culicine larvae. The second and third instars showed minimum body length when fed with anopheline larvae and fish fingerlings. The fourth nymphal instar showed minimal body length when fed with aedes larvae and the fifth nymphal instar with anopheline larvae. Measurement of individual organ was subjected to statistical analysis of variance. The rostrum length, head width, 1st leg, 2nd leg and 3rd leg showed statistically significant differences among the members of third nymphal instars that were fed on different test food. At other nymphal stages such differences were not observed.

3.3 *Food utilization in the post embryonic development*

Data on energy budget of nymphal instars of *D. indicus* when fed with different test

Table 1. Duration (in days) of the nymphal instars of *D. indicus* fed with different food types.

	Quality of food provided				
Nymphal instar	Mixed diet	Culicine larvae	Anopheline larvae	Aedes larvae	Fish fingerlings
I	8·7 ± 0·49	4·9 ± 0·49	6·0	6·1 ± 0·35	5·4 ± 0·53
II	8·7 ± 1·29	5·9 ± 0·7	5·0 ± 0·33	5·55 ± 0·74	4·5 ± 1·12
III	10·6 ± 2·11	8·6 ± 2·07	12·1 ± 3·84	11·73 ± 1·94	6·47 ± 1·51
IV	24·42 ± 1·0	22·42 ± 1·0	11·0 ± 2·56	23·33 ± 5·75	7·55 ± 0·62
V	37·67 ± 6·06	35·33 ± 6·62	18·36 ± 1·8	11·27 ± 3·0	13·0 ± 1·61
	90·09	77·15	52·46	57·98	36·92

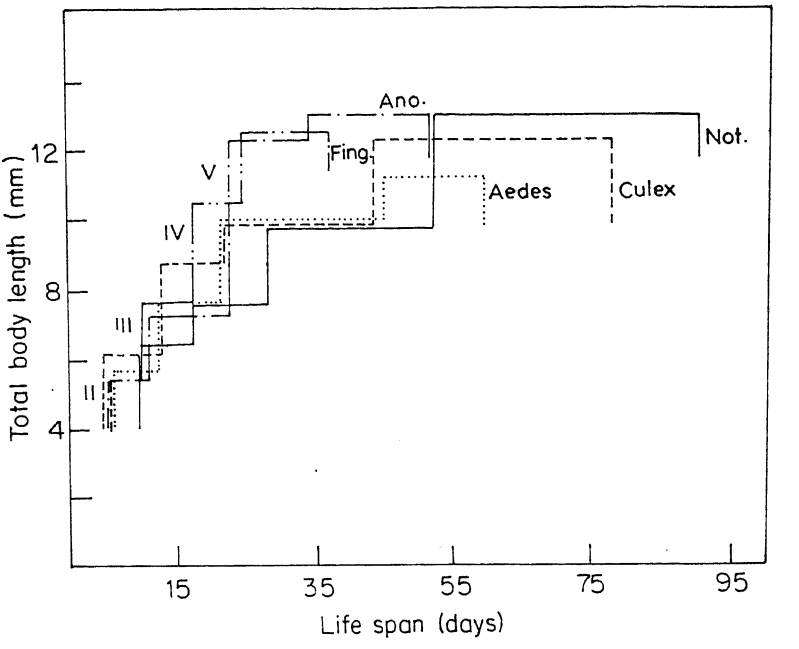

Figure 1. Mean body length against mean duration of nymphal stages of *D. indicus* fed with specific and mixed diets.

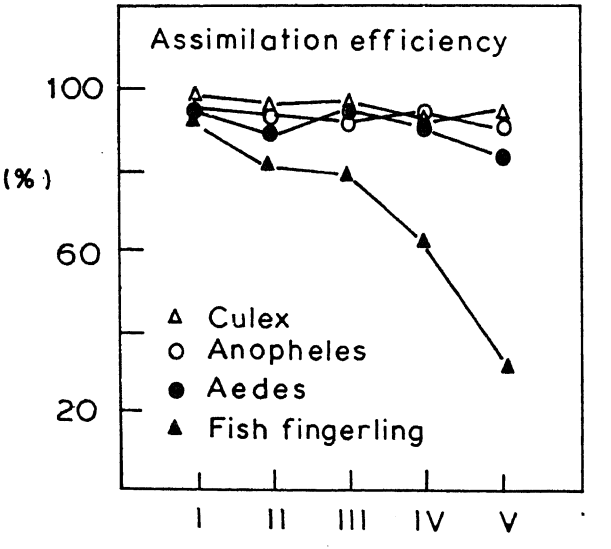

Figure 2. Effect of specific diet on the assimilation efficiency of *D. indicus*.

foods were recorded. In general, assimilation efficiency was almost 90% in the first nymphal instars of the predators and remain constant with others. In particular, assimilation efficiency of fifth nymphal instar of the predator was 82% with aedes larvae, 90% with anopheline larvae and 93% with culicine larvae (figure 2); whereas

the conversion efficiency at all stages of development was almost constant except the fifth nymphal instar but it is inversely related to the assimilation efficiency when fed with fish fingerlings (figure 3). The feeding rate was minimum when fed with fish fingerlings and maximum with culcine larvae. Among all nymphal instars, the second one showed the highest rate of feeding with culex and anopheles larvae. However, the feeding rate declined in other developmental stages (figure 4). The assimilation rate was almost equal in those fed on culex larvae, anopheles larvae or fish fingerlings; but those fed with aedes larvae showed 2 peak values with the first and third nymphal instars (figure 5). Similarly, the conversion rate was also noted to vary in relation to the quality of food provided, being higher at the second nymphal instar

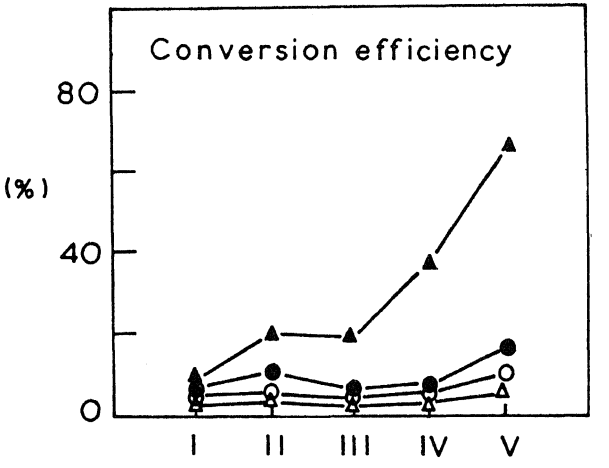

Figure 3. Effect of specific diet on the conversion efficiency of *D. indicus*.

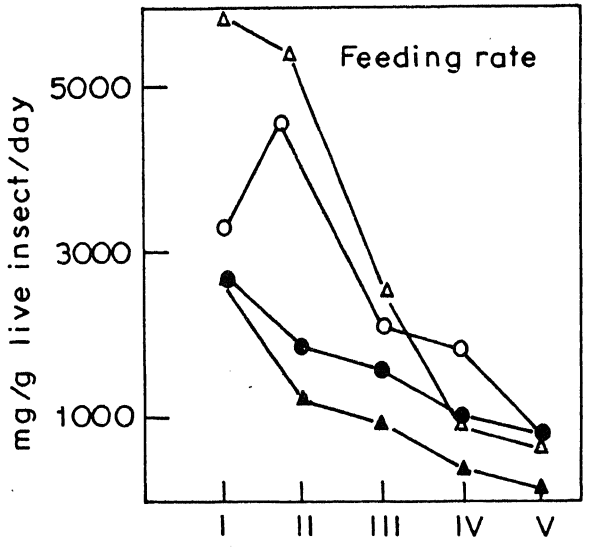

Figure 4. Effect of specific diet on the feeding rate of *D. indicus*.

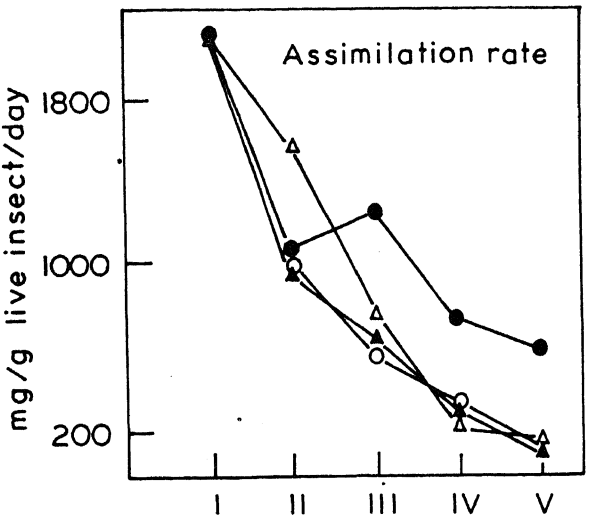

Figure 5. Effect of specific diet on the assimilation rate of *D. indicus*.

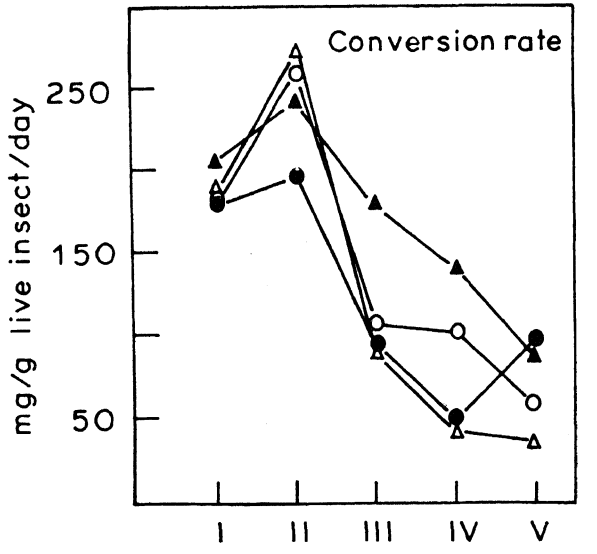

Figure 6. Effect of specific diet on the conversion rate of *D. indicus*.

when fed with larvae of *Culex*, *Anopheles* or fish fingerlings. It was considerably high when fed with fish fingerlings at all stages (figure 6).

Data on the energetics of the predator stages throughout their feeding periods is summarised in table 2. It may be noted that the feeding rate was minimum (1060 mg/g live insect/day) in fingerling fed individuals and very high (4300 mg/g live insect/day) in those fed with *Culex* larvae but the assimilation rate was maximum in *Aedes* fed ones and minimum when fed with *Anopheles* or fish fingerlings. The conversion rate was highest (171 mg/g live insect/day) when fed with fish fingerlings

Table 2. Energy budget of the nymphal instars of *D. indicus* fed with specific diet.

	Quality of food			
	Culicine larvae	Anopheline larvae	Aedes larvae	Fish fingerling
Duration (days)	77·15	52·46	57·98	36·92
Feeding rate (mg/g-live insect/day)	4300	2532	1550	1060
Assimilation rate (mg/g live insect/day)	955	841	1170	845
Conversion rate (mg/g live insect/day)	128	143	124	171
Assimilation efficiency (%)	95·2	92·8	89·6	68·8
Conversion efficiency (%)	5·0	7·0	9·6	29·4
Calorific value of the converted food (g cal)	234·85	185·81	115·1	154·9

Table 3. Energy budget of adult (male and female) *D. indicus* fed with culicine larvae.

Sex	Conversion efficiency	Assimilation efficiency	Feeding rate	Assimilation rate	Conversion rate
Male	20·36 ± 2·62	47·28 ± 2·66	497·24 ± 65·14	233·81 ± 17·67	45·6 ± 5·85
Female	18·75 ± 4·25	38·92 ± 5·03	558·11 ± 140·08	213·09 ± 34·74	41·98 ± 9·54

and lowest (124 mg/g live insect/day) in *Aedes* fed ones. The calorific value of the converted body tissue was 234·859, 185·81, 115·1 and 154·9 g/cal with *Culex*, *Anopheles* and *Aedes* larvae and fish fingerlings respectively.

3.4 *Energy budget in adult*

Table 3 shows that the assimilation efficiency of the male was 47·28% which is higher than that of the female. Similarly, the conversion efficiency was 20·36 in males and 18·75 in females but the feeding rate was low in males than in females. However, the assimilation and conversion rates were noted to be higher in males than in females.

3.5 *Egg viability in Ranatra*

The hatching success of eggs deposited by *R. filiformis* was recorded (figure 7). There was a great difference between the number of eggs laid among the batches. The number of eggs in 4 batches was 7·86, 9·93, 4·07 and 0·14 respectively. Also, the time interval between any 2 batches of the eggs varied significantly. The percentage of hatchability ranged from 64–82 in these batches. Data shows that the time interval between batches of eggs and percentages of hatchability were found to be inversely proportional with first and the last batch of eggs. Of the eggs laid in each batch, the hatching success was 74%, 62%, 78% and 84% respectively.

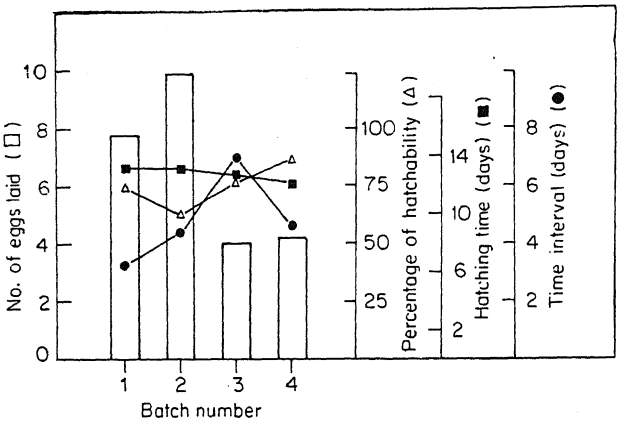

Figure 7. To show the hatching process of eggs oviposited by a female *R. filiformis.*

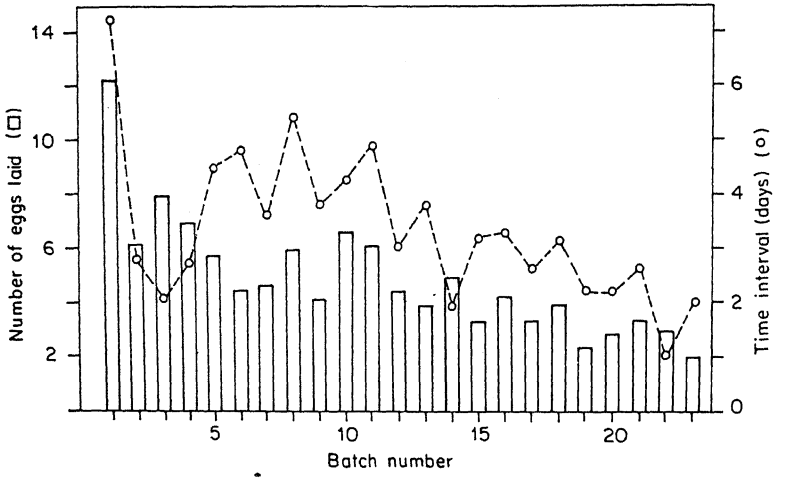

Figure 8. To show the rate of oviposition by a female *R. filiformis.*

3.6 *Rate of oviposition in Ranatra*

The number of batches of eggs laid by a female during its life time ranged from 17–23. The number of eggs laid was from a maximum of 12·33 ± 4·4 in the first batch to a minimum of 2 in the 23rd batch (figure 8). However, the trend in the fluctuation of number of eggs deposited batchwise during the life span of *Ranatra* was not highly significant; whereas the time interval between successive batches of eggs laid was significantly high. Generally, the hatching period was noted to be more or less constant. The bug took 5·987 ± 3·585 min to get acclimatised for oviposition. Time taken for actual oviposition of any egg in one batch ranged from 0·56–1·17 min. However, the time interval between successive eggs laid increased from the first to the last egg deposited (figure 9). When the percentage of success of successive stages of development and the percentage of success of development of egg to adult were

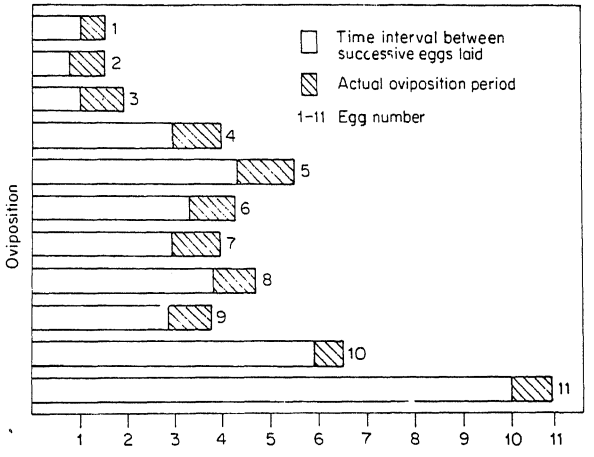

Figure 9. Time interval between successive eggs laid, actual oviposition period and number of eggs laid by a female *R. filiformis*.

Table 4. Success (%) of successive stages of development and of egg to adult in *R. filiformis*.

	Percentage success					
	Egg to I	I to II	II to III	III to IV	IV to V	V to Adult
Stages of development	89·0 ± 5·95	91·96 ± 2·65	81·36 ± 3·60	78·42 ± 4·80	74·78 ± 4·70	19·67 ± 3·53
Egg to adult	89·0 ± 5·95	81·80 ± 5·4	66·60 ± 5·8	52·40 ± 6·92	39·20 ± 6·08	7·80 ± 2·08

calculated, it was noted to be more or less constant, till the fifth nymphal instar but there was a sudden decline from fifth nymphal instar which was recorded as 19·6% irrespective of the sex moulted (table 4).

4. Discussion

Aquatic insects exhibit the ability to use a wide variety of food sources, exploit and develop them into a variety of macro- and microfeeders that operate from deep within the substrate to the top of the surface film (Resh and Solem 1978). Results of the present study reveal the ability of belostomatid bugs feeding on variety of prey items. However, a preferential selection was noted which has a direct influence on their longevity. Probably, prey mobility and morphology may suppress the predatory efficiency of the bugs concerned. Difference in mobility between *Ephemerella altana* and *Bactis tricaudata* was pivotal in producing the higher consumption of the stoneflies (Molles and Pietruszka 1983).

Rate of predation and food quality were observed to alter the duration of post-embryonic development in *D. indicus*. Clark (1963) has shown that some strains of *Habrobracon* females when fed on white cloves honey, lived much longer than *Ephestia* fed females. But in other strains, the adult life span was identical on either diet. Report of Alpatov (1930) on *Drosophila* and Strong and Kruitwagen (1969) on

Lygus support the present observations of the impact of nutrition on the life span and ageing of insects.

The results of energetics of developmental stages of *D. indicus* in relation to food quality show many interesting observations. When feeding is greater with *Culex* than with *Aedes* larvae, the conversion and assimilation efficiencies do not show much difference but with fish fingerlings, the conversion and assimilation efficiencies during development differed significantly. Such a feature is not uncommon in herbivorous insects as is known from the report of Bailey and Mukerji (1976) on *Melanoplus sangrinipes* and Smyth (1962) on *Anulis mali*. However, there appears to be no relationship between rate of feeding with that of rate of assimilation and conversion. But the finding that the high conversion rate of second nymphal instar that fed on culcine larvae is reflected in the increased body size of the penultimate instar is of considerable interest. Also, relationship between conversion rate and allometric growth including total body length was noted. This may be due to the difference in the nutritive requirements responsible for the growth of various organs (Kasting and Mcginnis 1959; Rockstein 1964).

Results on egg viability of *R. filiformis* show that the number of eggs laid in earlier batches is more than that in the later ones. The prolonged period of mating in the early period might have resulted in such increased oviposition and on experience, the females might have avoided repeated copulation which is well reflected in the minimum number of eggs laid in the later batches. Siew (1966) in *Galeruca tenaceti* has suggested that ovipositing females possess neurosecretory cells that produce more neurohormones resulting in an increased production of batches of eggs than do those of maturing females which further supports the present view that oviposition is frequent at a later stage irrespective of the number of eggs laid in each batch.

Results further indicate that the time interval between oviposition of successive batches of eggs is less in the later batches. This may reflect on the ability of females to store sperms (Parker 1970) and ageing (Spence and Scudder 1980). Data on ovipositional period show that the bug takes a minimum time to lay the eggs in the beginning and a maximum period at the end in a single batch. Demand for floating vegetation, energy lost during oviposition and the mobilisation of the matured eggs from the terminal part of the ovariole may be attributed as the causal factors for such variations in oviposition period during oviposition of eggs in a single batch. However, such an interpretation needs to be confirmed based on histological and histochemical studies.

Among the successive instars, a sudden fall is noted in the percentage of emergence of adults from fifth nymphal instar. This may be owing to an increased rate of mortality during the moulting of adults from fifth nymphal instar. This in turn may be either owing to the influence of increase in temperature that can eliminate a species by disrupting emergence patterns as is suggested by Nebeker (1971) in *Pteronarcys dorsata* or to the susceptibility of low oxygen concentration in the medium in other aquatic insects (Nebeker 1972).

The quantitative nutritional approach consists of measuring the amount of food consumed, digested and assimilated, excreted, metabolised and converted into biomass (Woodring 1979). Analysis of these measurements in *D. indicus* and the impact of nutrition on oviposition strategy of *R. filiformis* reveal how organisms respond to different foods and which food component exerts the greatest effects on growth. Further investigations on mobilisation of biochemical components from

haemolymph to various body parts may highlight the significance of food quality on reproductive biology of water bugs.

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Observations on feeding propensities, growth rate and fecundity in mayflies (Insecta: Ephemeroptera)

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Abstract. The relative importance of organic detritus and algae in promoting the growth and fecundity of some species of Baetidae is assessed on the basis of the nutritional differences among them. Taking into consideration the ephemeral aspect of adult life, an understanding of the relative importance of such substances as carbohydrate, protein and lipid in relation to their growth and fecundity is also examined.

Keywords. Feeding propensities; growth rate; fecundity; alga; detritus; mayflies.

1. Introduction

A basic facet of the structure and function of a freshwater ecosystem is the material cycling and energy flow. In turn, a significant portion of such cycling and flow involves the processing of various forms of organic matter by freshwater invertebrates, especially insects. This constitutes a basis for interest in trophic relations of aquatic insects (Cummins 1973). The nymphs of Ephemeroptera do not play the same part in the trophic structure of the communities in which they occur, and in view of this detailed knowledge of their feeding propensities is highly desirable.

The majority of mayfly nymphs are herbivorous, feeding on detritus and periphyton. The herbivorous mayflies fall into two main categories: collectors and scrapers (Edmunds 1978). Among the collectors, several genera are filter-feeders, with setae on the mouthparts or forelegs acting as filters. Within the Oligoneuridae, Leptophlebiidae, Siphlonuridae and the Heptageniidae, there are several genera that are probably filter-feeders (Wallace and Merritt 1980). By using their gills to produce a current of water through their burrows, several of the Ephemeridae and Polymitarcyidae may, at least for part of their food supply, be regarded as filter-feeders (Brittain 1982). Most mayflies, however, are fine-particle detritivores. These include many Siphlonurinae, Baetidae, Leptophlebiidae, Metretopodidae, Ephemerellidae, Caenidae and Baetiscidae, as well as some Heptageniidae (Edmunds 1978). The other major feeding group within the mayflies, the scrappers, utilize the periphyton present on mineral and organic surfaces. These include representatives of several mayfly families, notably the Baetidae, Heptageniidae, Leptophlebiidae and Caenidae (Edmunds *et al* 1976). Shredders are probably also represented among mayflies.

Earlier observations reveal that even within the detritivore/herbivore category, diet may change with season, habitat and stage of development. Seasonal differences are often a reflection of food availability (Brown 1961; McClure and Stewart 1976; Moore 1977), thus emphasizing the opportunistic nature of mayfly nutrition. However, within the range of food available, there is often evidence of selection (Brown 1961; Cianciara 1980). Food selection may clearly be advantageous, as growth may be influenced by different kinds of food (Anderson and Cummins 1979; Cianciara 1980; McCullough *et al* 1979).

Reproduction in insects is very closely related to nutritional factors, the qualitative and quantitative aspects of which have an impact not only on fecundity, but also on the rates of growth and development. Notable publications in this direction include those of Johansson (1958), Slansky (1980a, b), Ananthakrishnan *et al* (1982) and Raman and Sanjayan (1983). Whereas there are some investigations on feeding propensities (Brown 1960, 1961; Gupta and Michael 1981; Venkataraman 1984), food habits (Baekken 1981), trophic relations (Winterbourn 1974), food preference and dependence of growth on the type of food (Cianciara 1980) and nutritional dynamics (Zimmerman and Wissing 1980), work on the impact of nutrition on reproduction of mayflies is conspicuous by its absence. The present investigation aims at understanding and comparing the feeding propensities, growth rate and fecundity of two species of *Baetis* and one species of *Cloeon* available in and around Madurai, based on laboratory observations.

2. Material and methods

Early instar nymphs of *Baetis* sp. A and B were collected from Vaigai river near Thiruvedagam, 10 km west of Madurai. Early instar nymphs of *Cloeon* sp. were also collected from the temple tank of Thirumohur, 10 km north of Madurai. These nymphs were acclimated to the laboratory conditions (temperature $26 \pm 1°C$) for 24 h. Nymphs of constant length (approximately 2·0 mm), which are in the same physiological age were assorted. Twenty nymphs of each species were reared individually in separate petridishes of 9 cm diameter. Mortality was around 50% and dead nymphs were replaced by the nymphs of the same physiological age. A batch of 10 nymphs of each species was fed on crushed alga (*Spirogyra* sp.) and the remaining batch of 10 of each species on detritus collected from respective habitats. The two types of food (organic detritus and alga-*Spirogyra* sp.) used in the cultures were selected on account of abundant occurrence in the natural environment and of being well known from the literature (Brown 1960; Minshall 1967; Cianciara 1980). Food was supplied in excess. Filtered water brought from their natural habitats was used for rearing. A fresh supply of food was added every day when the water was changed. The head width and the body length of each nymph excluding antennae and cerci were measured after every moult. Since adult mayflies usually live for only a day or two and all the eggs are produced prior to the subimago stage (Clifford and Boerger 1974) total potential fecundity was determined accurately by counting the eggs in subimagos soon after emergence. The alga (*Spirogyra* sp.) and detritus were quantitatively analysed for proteins, carbohydrates and lipids by following the methods of Lowry *et al* (1951), Dubois *et al* (1956) and Kok (1971) respectively.

3. Results and discussion

It is evident from table 1 that *Baetis* sp. A and B grow faster in detritus than in alga (*Spirogyra* sp.) whereas it is vice versa in *Cloeon* sp. Apparently present observations indicate preference of detritus by the two species of *Baetis* under investigation and preference of alga by *Cloeon* sp. These are in conformity with the observations of some previous workers. Studies of Badcock (1949), Brown (1961) and Baekken (1981) on *Baetis rhodani* and of Gupta and Michael (1981) on *Baetis* sp. in Shillong,

Table 1. Growth rate of different species of mayflies fed on detritus and alga.

	Detritus		Alga	
	Body length (mm)	Head width (mm)	Body length (mm)	Head width (mm)
Baetis sp. A.	0·36 ± 0·08	0·15 ± 0·01	0·27 ± 0·08	0·10 ± 0·01
Baetis sp. B.	0·50 ± 0·05	0·14 ± 0·01	0·33 ± 0·03	0·09 ± 0·004
Cloeon sp.	0·20 ± 0·03	0·09 ± 0·01	0·66 ± 0·15	0·14 ± 0·01

Table 2. Quantitative profile of carbohydrate, protein and lipid as well as calorific values of different types of food.

	Carbohydrate (mg/100 mg)	Protein (mg/100 mg)	Lipid (mg/100 mg)	Calorific value (cal/mg)
Alga	5·1 ± 0·42	43·5 ± 6·3	11·2 ± 0·8	4,100
Detritus	1·09 ± 0·16	17·8 ± 0·35	8·2 ± 0·6	3,950

Meghalaya revealed them to be mainly detritivorous whereas Brown (1960) has shown that several species of algae were thoroughly and rapidly digested by *Cloeon dipterum* in the laboratory.

It is a well known fact that the type of food may have a substantial effect on the growth and fecundity of an organism. This is especially so in the nymphal span of mayflies whose adults are ephemeral and do not feed. Quantitative analysis of alga and detritus given as food for nymphs reveals that alga (*Spirogyra* sp.) is richer in carbohydrates, proteins and lipids when compared to detritus (table 2), though the calorific values of both types of food are approximate [*Spirogyra*—4,100 cal/mg, Ivanova (1958); detritus—3,950 cal/mg, Coffman *et al* (1971)]. When the nymphs of mayflies are feeding upon filamentous algae, the mandibles play a more important part in the collection of food than when this is fine detritus. Apparently the mandible tips of *Cloeon* sp. are more suitable for algal diet than those of *Baetis* spp. However, previous studies have shown little or no cellulose activity in mayflies (Monk 1976). Nevertheless, organic compounds leaked or secreted by the algae may be of nutritional importance (Cummins 1973).

The retarded growth of *Baetis* sp. A and B reared on algal diet in spite of its richness of nutrients may be correlated with their inability to consume large quantities of algae. The tip of the mandible of *Baetis* sp. A and B is more tapered than that of *Cloeon* sp. and the straight canines project towards the substratum rather than into the preoral cavity. Obviously the molar surfaces of *Baetis* spp. and of *Cloeon* sp. show constant differences in the details of their structure correlated with the nature of their respective diet though the feeding mechanisms of *Baetis* spp. and *Cloeon* sp. are adapted to allow the ingestion of a heterogenous diet in a variety of habitats as pointed out by Brown (1964).

It is also obvious from table 3 that the difference in fecundity of *Cloeon* sp. fed on alga and detritus is much more conspicuous than those observed in *Baetis* sp. A and B. Moreover, the quantity of food ingested also is of value in improving fecundity. Apparently it is the larger quantity of algae ingested by *Cloeon* sp. and the considerable quantity of detritus ingested by *Baetis* sp. A and B that might have

Table 3. Mean number of eggs/individual in different species of mayflies in relation to the type of food.

	Detritus	Alga
Baetis sp. A.	303 ± 1·41	245·5 ± 7·77
Baetis sp. B.	200 ± 2·82	151 ± 3·0
Cloeon sp.	101·5 ± 2·12	539·6 ± 33·2

resulted in increased fecundity of alga-fed *Cloeon* sp. and of detritus-fed *Baetis* sp. A and B in the present investigation.

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Impact of chemicals on feeding and reproduction in insects*

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Abstract. Pest control is today an important segment of entomology supported by objective programs of research, education and business management. In the continuous search for newer strategies for pest suppression, certain unique ideas have emerged and concretized to definite principles and practice. The two themes which merit relevance and recognition here are the use of chemicals directed to interfere with feeding and reproduction in insects. The impact of chemicals derived from natural and synthetic sources has been well utilized by entomologists to suppress pests effectively either by inhibiting the gustatory stimulus or the reproductive potential.

Keywords. Feeding behaviour; antifeedants; alkylating agents; reproductive potential.

1. Introduction

Insect pest suppression is a basic need and several strategies have been employed. Chemicals which control the complex behaviour of insects in choosing and rejecting plants for food can be intricately designed and used to achieve a considerable degree of pest suppression. Manipulation by chemicals results in interference with the gustatory stimulus by modifying the sensory input of the insect eventually causing starvation death. Another approach is to control the future pest generations by chemically disrupting the normal insect reproductive process. The impact of certain chemicals on insect feeding and reproduction will be discussed in this paper.

2. Impact of chemicals on feeding

The intricate insect chemoreceptors surrounding the mouth region are hypersensitive to different chemicals present in the potential host plants and thus the insects have the capacity to discern the presence of phagostimulants or phagodeterrents (antifeedants). The chemical composition of plants is the fundamental basis for acceptance or rejection of host plants as food in the phytophagous insect. The insect feeding behaviour is governed first by the host plant recognition and orientation, second by initiation of feeding and continuation of that activity and third by cessation of feeding. An antifeedant will inhibit the insect feeding by blocking the gustatory stimulus and thus disrupting the activity of initiation and maintenance of feeding. Some of the secondary plant products function as chemical defences against polyphagous insects, and in the presence of these natural antifeedants insects may perhaps take to feeding on less distasteful weeds or eventually starve themselves to death.

Antifeedants have been known for many years. As early as 1928, Eulan New was used for moth proofing woollens. Later triphenyl phosphorium salts, Mitin FF were also successfully used for protection of woollen against feeding of moth larvae. A

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series of compounds were later screened and the compound 24,055, i.e. 4-(dimethyl-triazeno) acetanilide was found to inhibit feeding in army worms. Many chemicals came under investigation, including inorganic salts of sodium, potassium, calcium, ammonium, arsenic, antimony and tin, aromatic hydrocarbons, quinones and naphthoquinones, alcohols, aldehydes, ketones and amines, amino acids, carbohydrates, acids and bases, sesquiterpene lactones, oils, limnoids, quassinoids, saponins, flavonoids, isoflavonoids, phenols, tannins, coumarins and furocoumarins, alkaloids, steroids, terpenes, terpenoids and organometallic fungicides and most of these were found useful in inhibiting feeding in various leaf eating and sucking pests. Certain insecticides also proved to possess antifeedant activity to some extent.

Our investigations showed that those chemicals which exhibited strong antifeedant activity induced similar patterns of behaviour in the test insect *Achoea janata* larvae. After the first few bites of the treated leaf discs the test insects were deterred from feeding. At no stage did the insect orient away from the treated leaf discs and this condition is a prerequisite for development of a good antifeedant. *Catharanthus* root and leaf alkaloid exhibited absolute antifeedant activity against *Achoea* larvae. So was the case with methanol extracts of *Nerium* leaves. Meisner *et al* (1981) also found that the leaf extracts of *C. roseus* were effective antifeedants against *Spodoptera.* Ascher *et al* (1980, 1981) showed that certain ergostane-type of steroids, with anolides isolated from solanaceous plants had a considerable anti-feedant effect to *Spodoptera.* Coumarins are already known as good antifeedants (Chapman 1974; Koul 1983). A compound structurally similar to coumarin known as 6,7, dimethoxy isochroman-3-one showed relative antifeedant activity (Reena *et al* 1983). Triphenyl tin acetate a well-known antifeedant proved very effective against *Achoea* larvae and also against nymphs of *Heiroglyphus banian.* Joshi *et al* (1971) and Raghupathy (1973) have shown triphenyl tin acetate as a good antifeedant against *Prodenia litura* and *Pericallia ricini.*

The mechanism of perception is dependent on the relevant receptors which differentiate stimulatory and inhibitory materials. Caterpillars have been shown to respond to inhibitory chemicals at several points in the feeding behaviour sequence and various ablation experiments have shown that sensilla on the maxilla are particularly important in perception of inhibitors not excluding other receptors. Dethier (1937) found that the response of caterpillars to odours was eliminated after removal of the antennae and maxillae and maxillary palps and the larvae of *Sphinx liquistri* after ablation fed on oleander and cabbage which were otherwise normally rejected. Removal of the palps from the larvae of *Leptinotarsa* also resulted in increased intake of normally unacceptable plants (DeWilde 1958). Fentin acetate an established antifeedant ceased to deter feeding in *Spodoptera* larvae by acting on the sensory organs situated on or near the mouth; this was proved by maxillectomy and extirpation of the labrums and antennae (Ascher and Ishaaya 1973). Kennedy (1967) and Ma (1972) interpret the results involving maxillectomy as indicating that the normal effect of the maxillary palpi of caterpillars is perhaps to inhibit feeding probably spontaneously and their removal disposes off this source of inhibition, while sensilla on other areas of mouthparts are responsible for the perception of inhibitory chemicals. The occurrence of other receptors of inhibitory materials elsewhere on the mouthparts was recognized by Ito *et al* (1959), Waldbauer (1962) and Ma (1969) since the intake of distasteful materials was not sustained and the insects were still able to differentiate degrees of distastefulness following

maxillectomy. All experiments thus far conducted indicated that the maxillae and especially the palps are particularly important in the recognition of inhibitory chemicals although the receptors elsewhere on the mouthparts are also involved.

The fact that insects may be prevented from feeding and damaging crops by interfering with their sensory inputs gains tremendous significance in the future pest control programme.

3. Impact of chemicals on reproduction

Insect reproduction is invariably influenced by many chemicals. By manipulating the dispersion of these chemicals during times detrimental to the insect, effective control can be established.

3.1 *Chemosterilants*

Chemicals which directly affect the reproductive system bringing about sterility in insects by interfering and interrupting the steps in the reproductive cycle are usually known as chemosterilants. As early as 1942, it was reported that Mustard gas caused sterility in *Drosophila.* Then followed chemicals showing similar action, wherein ethylene glycol, nitrogen mustard, formaldehyde and tretamine also were reported as reproductive inhibitors. Attention was soon diverted to specific groups of chemicals and now presently the chemosterilants could be fairly categorised into 3 groups (i) alkylating agents, (ii) antimetabolites and (iii) miscellaneous chemicals. We confine our discussions mainly only to these chemosterilants and to certain chitin synthesis inhibitors although hormones and ecdysones also interfere with insect reproduction.

3.2 *Physiological effects on the female*

Although the female insect presented a more complex reproductive system, most of the research on the physiological effects by chemosterilants was carried out on females. Since the newly emerged female possessed a partially developed ovary, the interference of chemicals with the process of maturation and Oogenesis was more observable. The first investigations in this aspect could be attributed to Goldsmith *et al* (1948), when they found the inhibition in ovarian development in *Drosophila* due to chemical interferences, and later many chemicals like amethopterin, methotrexate and 5-flurourasil were found to share this property of ovarian inhibition. Soon after LaBrecque (1961) reported a group of chemicals belonging to the alkylating group and commonly known as Apholate, Tepa and Metepa showing exceptional promise in affecting both sexes.

The most often observed physiological effect on female insects due to the chemical interferences was the retardation or complete cessation of ovarian development. The changes produced ranged from almost complete necrosis and disappearence of the organ to a slight reduction in size. The dose undoubtedly had a profound effect on the severity of damage. In general, the inhibition of ovarian development is the most outstanding characteristic feature of female chemosterilants particularly alkylating

agents and antimetabolites. Too many chemosterilants cause varying degrees of ovarian inhibition and it is impossible to discuss all. We will only see certain examples now, since the number and diversity of compounds that inhibit oogenesis is truly astounding. Some chemosterilants although caused sterility, did not affect the reproductive physiology adversely. We could not induce any adverse effects on the Hemipteran or Lepidopteran ovary with Hempa or Hemel even at very high doses but there are reports wherein Dipteran ovary showed retardation of growth. Some miscellaneous compounds like colchicine also inhibited ovarian growth. Numerous triphenyl-tin derivatives also disturbed the ovarian physiology.

3.3 *Physiological effects on the male*

The testis unlike the ovaries seldom show drastic morphological changes. Although certain alkylating chemosterilants have been reported to cause testicular atrophy but more frequently the treated male continues to produce seemingly normal motile sperm throughout their life. But the sperms in spite of its outwardly normal characteristics fail to produce adult progeny. The Zygotes present abnormal mitotic figures, chromosomal aberrations resulting in partial interrupted embryonic development. Certain chemicals like the S-triazines (Hemel) and melamine derivatives showed a male sex specificity in causing sterility. In certain cases the male accessory glands showed some inhibition in development when chemosterilants were administered at younger stages.

The chemosterilant effect is largely selective to the gonads. A direct interaction of the sterilant with the sperm and ovum has been demonstrated and this primary interaction seems to be the main mechanism of sterility (Borkovec 1969). Aziridines are known to inhibit various enzymes and also the synthesis of DNA of the gonads (Chamberlain and Barrett 1968; Madhukar *et al* 1971) as well as that of sterile eggs (Painter and Kilgore 1967). Ross (1962) showed that chemosterilants can cause cytostatic mutagenic and cytotoxic effects. Affected gonads exhibit various degrees of abnormalities. The spermatogenic activity may show disharmony and the direct interaction with the chemical can induce chromosomal breaks (Rai 1964). Such chromosomal anomalies find expression in dominant lethals in the embryo causing cell death (LaChance and Riemann 1964).

3.4 *Chitin synthesis inhibitors*

Chitin synthesis inhibitors mostly belonging to the group of benzoyl phenyl ureas were first developed as good insecticides around 1970 by Philips-Duphar Company. These compounds interfere with chitin biosynthesis during molting and hence are very effective as larvicides. Later, they were shown to possess a sterilizing activity on the adults. Diflurobenzuron commonly known as dimlin and its trifluroderivative, penfluron were the two chitin synthesis inhibitors which really gained immense popularity. In the case of these compounds also, the effect was more pronounced in females, than in males. Males perhaps transferred the compound to female during copulation which in turn acted as an ovicide. In certain Lepidopterans like nun moth and pink boll worm, adult treatment of benzoyl phenyl ureas presented no effects on spermatogenesis, mating or oviposition, but ovicidal effects were observed in codling

moth, Egyptian cotton worm, soyabean looper etc. Boll weevil adults treated with benzoyl phenyl ureas primarily showed reduced egg hatch and mating behaviour was affected and the size of the testis also got reduced. Ovicidal effects without adverse effects on fecundity was observed in hornflies, stable flies, houseflies, faceflies and screw worm flies. Penfluron initiated adverse physiological changes in both sexes of *Leptocoris* and *Dysdercus* (Satyanarayana *et al* 1985; Satyanarayana and Sukumar 1985).

4. Action of certain miscellaneous agents

4.1 *Anti-juvenile hormones*

Following the discovery of juvenile hormones (JH), hormonal research received an impetus and juvenile hormone-antagonists were envisaged. A JH antagonist will stop immature development resulting in a premature molt in an adult and an adult without JH cannot develop ovaries and would be sterile. Anti-JH are compounds which induced in insects precocious metamorphosis and Bowers named such compounds as Precocenes. Immature hemiptera undergo precocious metamorphosis by contact with precocenes. The resulting precocious adult females are sterile and the adult males are unable to inseminate normal females. Newly emerged females of cotton stainers and milk weed bugs treated with precocene show permanent sterility. But the application of exogenous JH to a sterilized female results in prompt ovarian development, showing that precocene acts to turn off the secretion of JH rather than interfering with JH action.

Interfering with the presence or absence of JH during developmental stage will be disastrous for insect reproduction. Precocenes prevented ovarian development or regressed the already developed ovaries. Apart from the precocenes initially derived from *Ageratum* certain synthetic compounds like benzyl-3-benzodioxole derivatives also proved to be good anti-JH compounds.

4.2 *Antibiotics*

Antibiotics also captured the attention of agricultural entomologists and many compounds were used for insect control.

Some of the ordinary solvents like benzene and acetone are reported to affect insect reproduction. Acetone at higher volumes 10 μl/insect reduced fecundity in some Lepidopterans, but not at lower volumes. Coming to some of the commonly used insecticides, carbaryl and carbofuran stimulated females to lay more eggs. Methomyl also increased the fecundity of females of diamond back moth.

It is already known that the Queen bee extract is found to reduce the fecundity of insects. Many amino acids tested against *Corcyra* showed promise as chemosterilants while certain other amino acids caused only partial sterility or at times increased fertility. Prostaglandins is also found to reduce eggs hatch and interfere with fecundity and fertility in cotton stainers.

Insect reproduction is a versatile area wherein the intricate mechanism causing insect sterility could be made use of in pest control programs.

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Impact of differential feeding on the reproduction of tiger beetle *Cicindela cancellata* DeJean (Cicindelidae: Coleoptera)

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Abstract. Tiger beetles belonging to the family Cicindelidae are exclusively predaceous and have great impact on the natural eco-balance. Recent studies have revealed the existence of more than 300 species all over India under divergent habitats and none have been exploited for biological control of insect pests. Studies on the impact of differential feeding on the reproduction of *Cicindella cancellata* revealed a definite relationship between the quality and quantity of food consumed by adults on the fecundity, egg size, longevity of the adults, larval emergence pattern and the survivability of first instar larvae. *Corcyra cephalonica* larvae fed 3 times a day (55·74 mg) resulted in maximum egg production, longer adult life and longer survival of first instar larvae, followed by Corcyra, one larvae per day (37·16 mg) and mixed food of spiders, hoppers and Corcyra (alternately) (32·81 mg). Least fecundity was observed when fed with ants alone and no eggs with no food.

Keywords. Tiger beetle; differential feeding; fecundity; survivability.

1. Introduction

Approximately around 2000 species of tiger beetles are known all over the world of which 300 species are reported from India. Almost all tiger beetles are predaceous both in their sedentary larval forms and mobile adult stage. Tiger beetles are generalists in their food habits and as such none have been exploited for biological control of insect pests. However, the major contribution of tiger beetle is as indicators of habitat degradation (Holeski and Graves 1978). The limiting factors in the distribution and abundance of tiger beetles in any ecosystem are oviposition site and food (Shelford 1908). The food intake rate of larvae is known to affect the size of later instars and adult size which in turn affect the individual fecundity (Hori 1982; Pearson and Knisley 1985). It is also stated that the years of abundant tiger beetles are normally the years of exceptionally high rainfall followed by the availability of rich prey. Therefore the study on the ecology and feeding behaviour assumes greater importance for the environmentalists.

The present study was aimed to know the adult food preference, and the impact of selective feeding on the reproductive potential and longevity of a commonly occurring riverbed tiger beetle *Cicindela cancellata* DeJean (Ganeshaiah and Belavadi 1986).

2. Material and methods

C. cancellata which occurs throughout the year on the river bed near sangam, 100 km from Bangalore, was collected periodically and the cultures maintained in the laboratory in small plastic containers measuring 7 × 12 cm. Newly emerged adults were confined in plastic containers, filled with 3/4 fresh sand. Optimum

moisture was maintained throughout the experiment at room temperature (23·65 ± 0·58°C). A male and a female was enclosed in each of the following 7 treatments and replicated 5 times. The treatments consisted of T_1 = one corcyra larva (37·16 mg) per beetle/day, T_2 = 3 corcyra larva (55·7 mg) per beetle, fed 3 times a day, T_3 = 4–5 ants (*Componotus compressus*, 77·0 mg) per beetle/day, T_4 = mixture of spiders, leaf hoppers and grass hopper nymphs (23·97 mg) per beetle/day, T_5 = one corcyra larvae (37·50 mg) per beetle once in two days, T_6 = mixture of hoppers and ants alternated with one corcyra larvae (32·81 mg) per day and T_7 = no food.

Actual quantity of corcyra larva fed was measured by weighing the beetles before and after feeding. The weight of beetles was recorded once before the commencement of the experiment and again on 12th and 18th day to know the difference if any. The rearing containers were changed on 9th and 20th day to avoid crowding of eggs/larvae. Corcyra larva head was crushed before feeding to prevent it from spinning. The weight of the ants and hoppers were recorded by freezing them for a few min before feeding.

Observations were taken for the number of eggs laid by counting the larval burrows everyday, even after the death of the beetles, till the last larval burrow was counted.

Female beetles were dissected to know the effect of differential feeding on the reproductive organs.

The data was subjected to statistical analysis following RCBD for longevity and fecundity, and correlation, regression for the rate of food consumed and survivability.

3. Results and discussion

It can be seen from table 1 that fresh weight of the female beetles at the beginning of the experiment ranged from 67·9 ± 5·08 to 77·98 ± 5·6 mg, and the males weighed 51·2 ± 6·08 to 58·56 ± 4·55 mg. No significant differences in the weight of the beetles was observed on the 12th and 18th day of experiment, compared to the weight before commencement of experiment. Initially a female beetle consumed 11–15 mg of prey (T_1, T_2 and T_6), whereas males consumed only 7–13 mg of prey, suggesting that the amount of food consumed depended on the size and body weight.

The egg laying extended upto 4 weeks in almost all treatments except in mixed feeding with hoppers and spiders, where the egg laying stopped in the second week itself (table 2).

The number of eggs laid was maximum in beetles supplied with 3 corcyra larvae (T_2) per day. The next best was one larvae per day (T_1) and mixed feeding (T_6) but statistically these 3 were on par and significantly different from those that were fed with ants (T_3), mixed feeding (T_4) and feeding with corcyra larvae on alternate days (T_5). Treatment of ants alone, mixed feeding and corcyra on alternate days were on par. Also treatments T_1, T_4, T_5 and T_6 were on par and superior to ants alone (T_3) (figure 1).

The number of eggs laid were directly related to the quantity and quality of food consumed by the adults (r = 0·96) (table 3). According to Pearson and Knisely (1985) adult females of 3 species of tiger beetles at a low feeding levels produced significantly lower eggs and larvae than females at higher feeding levels, further they found that

Table 1. Body weight of the beetles (n = 5) in relation to the quantity of food consumed during the study period.

		Initial weight (mg)	Av. food consumed at 2 days (mg)	Weight at 12 days (mg)	Av. food consumed at 12 days	Weight at 18 days
Corcyra (T_1)	Female	72·60 ± 7·6	14·50 ± 3·55	72·15 ± 5·21	9·30 ± 1·77	72·90 ± 7·15
	Male	51·20 ± 6·08	6·87 ± 2·16			
Corcyra (T_2)	Female	74·74 ± 4·20	13·94 ± 2·60	74·1 ± 2·01	11·00 ± 0·21	72·40 ± 5·00
	Male	56·22 ± 6·86	13·08 ± 2·99			
Ants (T_3)	Female	67·90 ± 5·08	—	73·10 ± 3·30	—	66·73 ± 2·56
	Male	58·56 ± 4·55				
Spiders, leaf and grass hoppers (T_4)	Female	69·44 ± 11·31	—	69·20 ± 15·56	—	61·36 ± 7·52
	Male	53·08 ± 3·87				
Corcyra once in two days (T_5)	Female	70·02 ± 5·12	—	66·00 ± 5·58	—	61·40 ± 9·15
	Male	53·88 ± 2·19				
Mixed and alternate with corcyra (T_6)	Female	77·98 ± 5·60	10·41 ± 2·68	69·50 ± 6·85	5·90 ± 5·36	61·66 ± 5·41
	Male	54·84 ± 6·64	10·52 ± 4·21			
No food (T_7)	Female	71·35 ± 4·80	—	69·50 ± 3·9	—	62·50*
	Male	56·28 ± 4·11	—	—	—	

*One observation

Table 2. Larval emergence holes of *C. cancellata* from the 14th day after enclosure.

Days after enclouser	14	16	18	21	24	27	34	45	46	48
Corcyra (T_1)	0	1·75	8·75	17·50	7·3	22·50	28·00	30·25	31·25*	31·75
Corcyra (T_2)	2·4	5·40	18·20	21·80	26·4	29·20	35·00	40·60*	40·60	
Ants (T_3)	0	0·33	2·66	3·33	4·0	5·00	5·00	6·00*	6·00	
Spiders, leaf and grass hoppers (T_4)	1·75	7·25	13·00	16·75	17·5*					
Corcyra once in two days (T_5)	2·20	7·20	8·80	14·80	17·4	21·40	22·80	23·40*	23·40	
Mixed and alternate with corcyra (T_6)	4·00	6·33	9·33	13·00	16·33	20·33	23·33	28·33*	28·33	
No food (T_7)	0	0	0	0	0	0	0	0	0	0

*Last larval emergence noticed.

small adult females produced fewer eggs and larvae at low feeding levels than larger conspecific females at the same feeding levels.

The longevity of the adults fed with ants was on par with no-food and the fecundity was least among the beetles that were fed (table 3). It is evident from this finding that the major constituents of the prey of tiger beetles in the riverbed is not ants as it was commonly believed. Irrespective of the quality and quantity of the food consumed and with and without food, the longevity of the male did not vary much (figure 2) whereas the longevity of the female seems to depend on the quality and quantity of food, females live for only 17·66 ± 1·80 days when starved compared to beetles fed with corcyra thrice a day (45·25 ± 7·46 days).

There was reduction in the size of the egg as well in the number of eggs in the

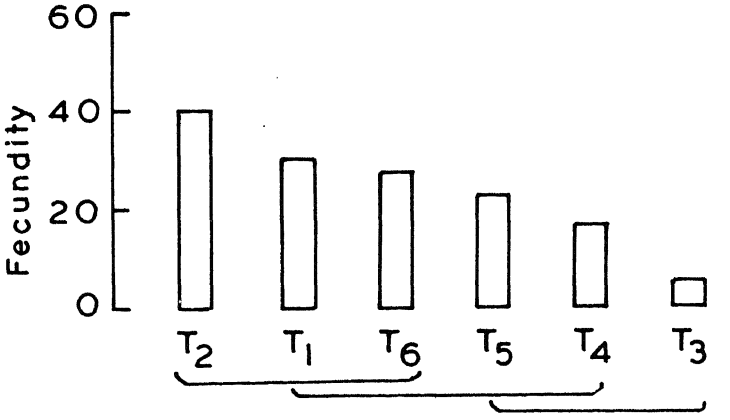

Figure 1. Fecundity of *C. cancellata* in different treatments. T_1, one corcyra; T_2, 3 corcyra; T_3, ants; T_4, mixed; T_5, alternate day corcyra; T_6, mixed + corcyra.

Table 3. Effect of differential feeding on the longevity of adults and survivability of larvae in *C. cancellata.*

	Quantity of prey given per day (mg)	Mean longevity (days)		Mean No. of larvae per female	Survivability of first instar after 60 days (%)	Moulting to second instar (%)
		Male	Female			
Corcyra (T_1)	37·16	11·75 ± 9·54	37·50 ± 6·03	31·33 ± 9·06	55·20	1·60
Corcyra (T_2)	55·74	8·00 ± 3·74	45·25 ± 7·46	40·60 ± 6·97	73·41	7·38
Ants (T_3)	77·00	16·00 ± 4·40	17·66 ± 4·11*	6·00 ± 1·41*	27·77	0·00
Spiders, leaf and grass hoppers (T_4)	23·97	9·50 ± 2·30	25·75 ± 4·96	17·5 ± 4·10	57·14	1·43
Corcyra once in two days (t_5)	18·50	8·00 ± 4·84	39·80 ± 12·74	23·40 ± 5·23	37·61	1·71
Mixed and alternate with corcyra (T_6)	32·81	12·33 ± 3·85	42·33 ± 20·00	28·33 ± 3·30	55·29	2·35
No food (T_7)	—	7·00 ± 1·41	17·66 ± 1·80	0	0	0
Correlation value		0·81**	0·814**	0·96**	0·39	0·282

*Correlation value excluding T_3.
**Significant.

follicle depending upon the duration of starvation. The initial mean number of eggs found was 8·0 ± 3·08 which reduced to 2·6 ± 2·57 in 5 days starved beetles and no eggs were found in completely starved beetles at 20 days.

Survival of first instar larvae was maximum (73·41%) in treatment T_2 (3 corcyra/day) followed by mixed feeding T_4 (57·00%), T_1 and T_6 treatment (55%). Evidently increased food to adult has increased the vigour and survivability of the larvae. It is also evident that the first instar larvae from the maximum fed adults (T_2) can pass to second instar without feeding as is seen in table 3.

Therefore quality and quantity of adult food intake has great influence on the number of eggs laid by the predator, on the longevity of adults, larval survivability and vigour.

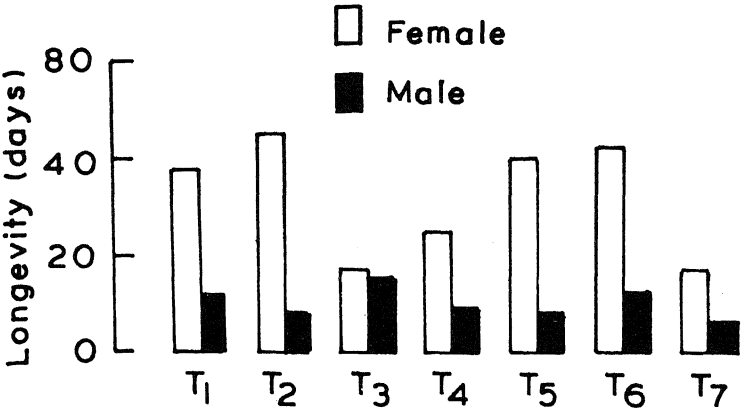

Figure 2. Longevity of male and female *C. cancellata* in different treatments. Same notations as in figure 1.

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Some observations on the nutrition—reproduction correlation in grouse locusts (Orthoptera: Tetrigidae)

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Abstract. The correlation between nutrition and reproduction with reference to two grouse locusts, *Euscelimena harpago* (Tetrigidae: Scelimeninae) and *Potua sabulosa* (Tetrigidae: Cladonotinae) is assessed. As far as *Euscelimena harpago* is concerned the impact of nutrition on reproduction is presented with reference to ovarian development in certain nymphal stages and in the adult. On the other hand, the impact is evaluated from an altogether different angle as far as *Potua sabulosa* is concerned. This pigmy locust prefers moss as food and tides over winter and hazardous summer as adult. During this period it almost does not feed. This peculiar feature is used as a parameter to assess the nature of impact in *P. sabulosa.*

Keywords. Nutrition; reproductive biology; insect-plant interaction; preferred and non-preferred host; Tetrigids (grouse locusts).

1. Introduction

The Tetrigids essentially are phytophagous caeliferan orthoptera, considered to be primitive and phylogenetically related to the acrididoid grasshoppers and locusts on the one hand and the tridactilids on the other (Paranjape *et al* 1986). Contributions by Bhalerao and Paranjape (1986), Gangwere (1961), Hodgson (1963), Hancock (1898), Paranjape (1976, 1985), Paranjape and Bhalerao (1985), Paranjape *et al* (1986) and Poras (1979) are the most significant ones while some acridological work with reference to sensillar pattern reported by Ananthakrishnan *et al* (1985), to plant chemistry and feeding behaviour by Bernays and Chapman (1978), and to host preferences as well as biochemical parameters by Sanjayan and Ananthakrishnan (1986) are of considerable importance in relation to the grouse locusts. As no information is available on the impact of nutrition on reproduction in these insects an attempt has been made to study these aspects with reference to *Euscelimena harpago* Serville and *Potua sabulosa* Hancock.

2. Material and methods

Samples of live *E. harpago* (Subfam.: Scelimeninae) and *P. sabulosa* (Subfam.: Cladonotinae) were periodically collected from localities in Pune and Mahabaleshwar. The grouse locusts were kept in cages containing their natural food. The female specimens were periodically dissected to study mainly, the development of ovaries with reference to the nature and the number of ovarioles, the oocyte-ovariole index and accumulation of fats in the body; after measuring the body length. The different measurements were taken using calibrated eye-piece gratings, of a zoom type trinocular dissecting microscope. Since *P. sabulosa*, the pigmy locust shows an altitude-limited

distribution (Paranjape and Bhalerao 1985), to verify the behavioural change, if any, during the overwintering period, these specimens were maintained in the laboratory in Pune before the onset of winter. They were also collected periodically from their natural habitat in Mahabaleshwar in Dist. Satara, the hill station located at an altitude of about 1573 m and about 120 km from Pune.

3. Results and discussion

3.1 *Food of grouse locusts*

The tetrigids are known to be feeding mainly on algae, bryophytes (especially moss: *Funaria* sps.), fungi and lichens, on detritous material and also on humus. *E. harpago*, a predominantly semi-aquatic grouse locust, shows preference to algae and detritous material (Bhalerao and Paranjape 1986) as is also revealed by the study of foregut contents. The scelimenids as shown by *E. harpago*, are known to be occasionally feeding on sproutings of paddy and also showing necrophagy. On the other hand, the pigmy locust, *P. sabulosa* shows its preference for moss (*Funaria* sps.) that luxuriantly grows in the said habitat with humid and rather cool climate, and also feeds on humus.

Although the grouse locusts possess the biting and chewing type or mandibulate type of mouth parts that are characteristic of orthoptera in general, the molar regions of the mandibles have been shown to be lacking in molar dentes that are present in other acridoids (Paranjape 1985). Instead, the SEM studies of the molar region in these insects (Paranjape *et al* 1986) have revealed an alternating grooved and serrated ridged pattern that is suitable for eating the soft, pulpy food. Moreover, this very pattern seems to be acting as a limiting factor when tough textured monocot leaves of cereals and grasses are considered as alternate host of these phytophagous orthopterans.

The habitat together with the climatic conditions in a year, availability of the food/host-plants and host plant preference by the two grouse locusts under study and the impact of these on their reproductive biology as is revealed by the observations done so far, are briefly given in the account to follow (table 1).

3.2 *Observations in E. harpago*

These grouse locusts are found in semi-aquatic habitat that also ensures their preferred food almost throughout the year excepting the summer months that are hazardous. *E. harpago* therefore shows ability to lay eggs almost from June through February/March in natural conditions that are available during major parts of the year. This is supported by the observation that one can find different nymphal stages, adults and periodically even gravid females, practically throughout the year. During the summer months however the population declines and consists of aestivating 5th/6th instar nymphs and a few adults. As far as the impact of food is concerned the findings are as under:

The zero day female adults when fed on paddy sproutings as an intermediate preferred food, died after 5/6 days. Furthermore, the maize plant turned out to be a

Table 1. Impact of seasonal variations on the ovaries of two grouse locusts.

	Stage	Month	Body length (mm) Mean	Nature of ovary	Ovariole No. Average	Oocyte—Ovariole index (Mean)
E. harpago	Nymph-III	Nov.	6·35	Panoistic, translucent	32	0·041
	Nymph-V	Nov.–Dec. (Winter)	9·04	Panoistic, translucent	38	0·057
	Zero day	Nov.–Dec.	22·56	Panoistic, light yellow	46	0·092
	Adult	Nov.	24·26	Panoistic, deep yellow	50	0·148
	Adult (Gravid)	Jan.	25·08	Panoistic, deep yellow	50	0·232
P. sabulosa	Adult (Lab.)	Nov.–Dec. (Winter)	7·50	Panoistic, almost transparent	24	0·110
	Adult (Nature)	Nov.–Dec.	8·00	Panoistic, almost transparent	23	0·142
	Adult (Gravid)	June (Monsoon)	8·20	Panoistic, deep yellowish brown	22	0·519

non-preferred host because the zero day female adults died in about 26 h. The modified mandibular molar region makes it difficult for these insects to feed on the said hosts, by acting as some kind of a limiting factor.

3.3 *Observations in P. sabulosa*

The pigmy locust on account of its habitat peculiarities and relative preference for moss-humus complex, presents an altogether different case for study. The gravid females (table 1) lay eggs in June–July. The hatchlings metamorphose and become adults by the following October. The adults are seen to be overwintering (November–December/mid January or so). During this period there is very little food intake, large quantities of fat accumulate in the body, while ovarial filaments show hardly any growth, save the large basal oocyte. The entire ovary appears transluscent indicating that the process of vitellogenesis as such is not active. The pigmy locusts kept in the laboratory from October through January showed similar ovarian development when compared with the specimens collected periodically from their natural habitat (table 1). During February through May, the adults try to overcome unfavourable summer conditions by burrowing in soil (Paranjape and Bhalerao 1985). During this period they practically do not feed. With the beginning of monsoon the ovarian development is activated and the adults copulate and lay eggs as stated earlier. Further the above aspects will throw more light on the impact of various environmental factors on the reproductive biology of the males and females of the pigmy locust.

4. Conclusions

(i) The studies carried out so far indicate that in the grouse locusts nutrition has an impact on reproduction.

(ii) As compared to acridoids the grouse locusts have relatively specific habitat and trophic requirements.
(iii) The peculiar modification of the mandibular molar region acts as an adaptation to the type of food that these insects prefer and at the same time as a limiting factor when it comes to their feeding on tough textured leaves.
(iv) Along with food the climatic factors also influence reproductive biology of these insects although the degree is different in the grouse locusts under study.
(v) The primitive phylogenetic status of the grouse locusts, their habitat and their peculiar feeding preferences on lowly evolved food plants, provides an interesting case for study from the point of view of plant-animal coevolution, since the importance of this topic has already been suggested by Swain (1978).

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nfluence of biochemical parameters of different hosts on the biology of *Earias vittella* (Fab.) (Noctuidae: Lepidoptera)

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Abstract. The present study aims to understand the influence of biochemical parameters of the host plants viz *Abelmoschus esculentus* L., *Gossypium hirsutum* L. and *Abutilon indicum* G. Den. on the reproductive biology of *Earias vittella*. The results indicate remarkable variation in the biochemical parameters of host plants which affected significantly the growth index and reproductive potential of the insect species. Decrease in body weight, fecundity, prolongation of larval period, percentage of larva becoming adult and adult longevity of both male and female was observed when reared on *Abutilon indicum* which had less amount of reducing sugars, protein and free amino acids but had high amount of non reducing sugars. Decreased larval period, increased fecundity and adult longevity were observed when reared on cotton and okra which had high amount of reducing sugars, free amino acids and protein. There was no significant difference in the amount of total soluble sugars among the 3 hosts tested. The growth index and fecundity of *Earias vittella* are apparently high on okra than on cotton. It is observed that high amount of reducing sugars, free amino acids and protein seems to be favourable for increase in fecundity of *Earias vittella*.

Keywords. *Earias vittella*; reproductive biology; okra; cotton; *Abutilon indicum*; biochemical parameters; incidence.

Introduction

he pest status of an insect species depends on its ability to breed on a variety of host lants, comparative growth rate, fecundity, population dynamics and distribution nanthakrishnan 1977). It was reported by Khan and Rao (1960) that preferred osts have a great influence on oviposition, fecundity, developmental period and ngevity of adults of *Earias vittella* (Fab.) and *E. insulana* (Boisd.). Mehta and axena (1970) were the first to study the role of ovipositional responses of *E. vittella* its establishment on different plants. Subsequently in 1973, they reported that rowth of *E. vittella* varies with the degree of consumption, nutritive value and tilization of food from different plants. The insect grows best on diet whose nutritive alue and degree of conversion into body matter are high. Krishna *et al* (1977) eported on oviposition in *E. vittella* in relation to adult nutrition, mating and some nvironmental factors. Vishwapremi and Krishna (1974) working on *E. vittella* rought to light the need for a carbohydrate diet, especially raffinose during its adult age for achieving maximum fecundity. Hiremath (1984) investigated the host reference of *E. vittella* on 7 different hosts under field conditions and the effect of esc host plants on development, longevity and fecundity. Mani *et al* (1986) studied e effects of larval food quality of okra and *Hibiscus rosasinensis* on egg output and ability in *E. vittella*. The present study aims to relate the biochemical nature of 3 host lants viz *Abelmoschus esculentus* L., *Gossypium hirsutum* L. and *Abutilon indicum* . Den. with the reproductive biology of *E. vittella*.

2. Material and methods

Three plant species viz *A. esculentus* (okra), and *G. hirsutum* (cotton) grown in fields and *A. indicum* found along the bunds in the campus of the Fredrick Institute of Plant Protection and Toxicology, Padappai, were taken up for investigations. From the laboratory culture of *E. vittella* adequate number of larvae were obtained for the studies. On each of the host plants viz *A. esculentus* (epicarp removed okra fruit), *G. hirsutum* (opened tender bolls of cotton) and *A. indicum* (opened young fruits), hundred freshly hatched larvae were released and the duration of larval and pupal stages, adult longevity and fecundity were also recorded. The studies were replicated thrice.

Simultaneously, samples of the larval food material were analysed for reducing sugars, total sugars, free amino acids and protein. Ethanol extracts of the larval food material were obtained by using 80% ethanol. The reducing sugars were determined first and subsequently the nonreducing sugars in the extract were hydrolysed to reducing sugars and the total sugars were estimated by Nelson's (1944) method. By subtracting the reducing sugars from the total sugars the nonreducing sugars were estimated and expressed as glucose equivalents. Free amino acids were estimated by Moore and Stein's (1948) method and protein by Lowry's (1951) method (Mahadevan and Sridhar 1982).

3. Results and discussion

The data presented in table 1 shows the developmental period, longevity, survival, weight of fourth instar larva, pupa and adult, fecundity and growth index of *E. vittella* on 3 hosts viz okra, cotton and *A. indicum*. The biochemical constituents of the larval food viz the seeds of the 3 host plants with regard to reducing sugars, nonreducing sugars, free amino acids and protein are furnished in table 2.

The duration of larval and pupal period was considerably longer on *A. indicum* being 18·33 ± 3·70 and 13·03 ± 3·11 days respectively and the toal developmental period worked out was 32·10 ± 2·98 days. On the other hand, the total developmental period was the shortest on okra (26·90 ± 3·20 days) while slightly more on cotton being 27·90 ± 3·12 days. The percentage survival has been found to be the lowest on

Table 1. Biology of *E. vittella* on three different hosts.

Host	Duration (days)			Total developmental period (days)	Survival (%)	Growth index*
	Incubation	Larva	Pupa			
Okra	4·57 ± 0·32	12·73 ± 2·41	11·16 ± 2·79	26·90 ± 3·20	68·00 ± 5·72	2·52 ± 0·21
Cotton	4·55 ± 0·41	13·76 ± 3·10	12·36 ± 2·54	27·90 ± 3·12	67·00 ± 9·09	2·40 ± 9·09
A. indicum	4·57 ± 0·38	18·33 ± 3·70	13·03 ± 3·11	32·10 ± 2·98	26·33 ± 3·29	1·02 ± 0·13

Host	Longevity (days)		Fecundity	Weight (g)		
	Male	Female		IV instar	Pupa	Adult
Okra	10·76 ± 2·20	14·60 ± 3·12	350·67 ± 28·77	0·084 ± 0·011	0·069 ± 0·005	0·024 ± 0·004
Cotton	9·33 ± 2·84	14·13 ± 3·09	301·66 ± 14·52	0·077 ± 0·014	0·068 ± 0·009	0·023 ± 0·06
A. indicum	6·23 ± 2·45	9·90 ± 2·98	135·00 ± 20·05	0·050 ± 0·013	0·045 ± 0·005	0·014 ± 0·003

Survival (%)

Table 2. Biochemical constituents* in seeds of three hosts of *E. vittella.*

Host	Reducing sugars	Non reducing sugars	Total soluble sugars	Free amino acids	Proteins
Okra	5·66	12·45	17·58	2·73	15·10
Cotton	5·88	13·34	18·62	2·65	14·64
A. indicum	1·74	16·38	18·12	1·85	12·02

*Values in mg/g (mean of 3 replications).

A. indicum being 26·33 ± 3·29 as against very high levels (67·00 ± 9·09 to 68·00 ± 5·72% respectively) on cotton and okra. The growth index was also found to be poor on *A. indicum* and was fairly high in okra and cotton. The fecundity, longevity of adults and the weight of fourth instar larva, pupa and adult on okra was comparatively higher than that of cotton. In these aspects there has been considerable reduction in case of *A. indicum.* The duration of larval and pupal periods, adult longevity and fecundity observed in the present study on okra, cotton and *A. indicum* are in conformity with the observations of Hiremath (1984). Vedamoorthy and Reed (1977) reported that the spotted bollworms *E. vittella* and *E. insulana* exhibits marked preference for various host plants. Vishwapremi and Krishna (1974) observed maximum fecundity in *E. vittella* when the larvae were reared on developing seeds of okra.

It is obvious from the present study that okra and cotton are the most favoured hosts. This finds correlation with the high level of reducing sugars, free amino acids and proteins and low level of nonreducing sugars. In the unfavourable host namely *A. indicum*, reducing sugars and free amino acids are very low. The nonreducing sugars are considerably high and protein content is also comparatively very less. However, there is no much variation in the level of total sugars. Though Mehta and Saxena (1973) studied the nutritive value of host plants, there is no quantitative indication of the various biochemical parameters. Only qualitative assessments have been presented. It is evident from the present study that a high level of reducing sugars, free amino acids and proteins and low level of nonreducing sugars in the food plants of okra and cotton favour high fecundity, survival and quicker development of *E. vittella* and these nutrients are found in less amount in the unfavoured *A. indicum.* In this connection it may be of interest to note that high level of reducing sugars in *Brassica campestris* has been correlated with high level of infestation of the agromyzid pests viz *Liriomyza brassicae* Rilay and *Chromatomyia horticola* Gour (Ipe 1985) and in rice increased amount of free amino acids was correlated with its susceptibility to yellow stem borer (Vidyachandra *et al* 1981). Though *A. indicum* was found in large numbers in the campus and throughout the state, periodical surveys revealed no natural infestation and the nonpreferred nature of the host might probably be responsible for this. In Karnataka, on the other hand infestation of *E. vittella* on *A. indicum* was observed and the mean number of fruiting bodies damaged was 0·10 as against 5·74 on cotton and 60·68 on okra.

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Effects of feeding regime on energy allocation to reproduction in the silkworm *Bombyx mori*

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Abstract. Pattern of energy allocation for reproduction was studied as a function of feeding regimes in the silkworm *Bombyx mori*. The larvae were restricted to feed for 6, 12, 18 or 24 h (*ad libitum*). Restriction of feeding duration resulted in slow growth and extended larval duration. Consumption, assimilation, conversion and metabolism and their rates showed steady increase with the increase in the feeding duration. On pupation, energy allocation for egg production was studied by dissecting the pupa and estimating the reproductive and body tissues separately during the entire pupal period. Length and biomass of ovary steadily increased with increasing feeding duration as well as with increasing age of pupa. During egg development, the tissue protein was transferred to the ovary. This type of conversion was more in those fed *ad libitum* than in those restricted to feed for shorter durations. Tissue fat and ovarian fat decreased as a function of age of pupa, indicating that fat served as the main source of energy for pupal metabolism. *Bombyx mori* restricted to feed for 6 h duration allocated 55% pupal energy for reproduction while those fed *ad libitum* allocated about 67% of the pupal energy for reproduction. However, they allocated about 15–19% of assimilated energy for egg production in all the feeding regimes.

Keywords. Silkworm; *Bombyx mori*; feeding duration; energy allocation; egg production.

1. Introduction

Food is a factor of paramount importance which regulates animal growth, reproduction and diversity of animals in nature (White 1978). Negative effects of diminished/restricted rations on food utilization in herbivores was perhaps first considered by Muthukrishnan and Delvi (1974). Subsequent publications in this area concentrated on the effects of restricted feeding on food utilization and growth of lepidopterous larvae (Mathavan and Muthukrishnan 1976; Muthukrishnan *et al* 1978; Muthukrishnan 1980; Christopher 1983). Surprisingly none of the investigators concentrated on the effects of feeding regime on the fecundity. Egg production depends on accumulation of sufficient nutrient during larval period and attainment of ideal adult weight; it will facilitate for the allocation of substantial nutrient energy by the adult for egg production (Engelmann 1970; DeWilde and DeLoof 1973a, b; Slansky 1980a, b; 1982). The present paper reports on the effects of restriction of feeding duration on food utilization and energy allocation for egg production by the silkworm, *Bombyx mori*.

2. Materials and methods

Fourth instar larvae of *B. mori* ($TN \times NB_4D_2$) were obtained from the Sericulture Department, Madurai. They were brought to the laboratory, acclimated and reared in cellular trays. Freshly moulted final instar larvae were used for the experiment. The larvae were divided into 5 groups consisting of 100 larvae each. Larvae in the I

group were restricted to feed for 3 h/day; those in the II, III, IV and V groups were allowed to feed for 6, 12, 18 and 24 h/day respectively. Triplicates were maintained in each group. Faecal pellets were collected separately for each tray during the time of removal of unfed leaves (once in a day) and were oven dried at 90°C for weight constancy. Prior to spinning, few worms from each group were sacrificed for dry weight determination and the remaining worms were allowed to pupate.

A minimum of 10 female pupae were identified from each series on every alternate day. The above collected pupae were weighed and dissected; the reproductive (ovary) and vegetative tissues (body tissue) were carefully separated. Ovary length, egg number and weight of the ovary were measured and dried separately. The powdered dry ovarian and body tissues were analysed for their chemical composition. Protein was estimated following the method of Lowry *et al* (1951) and that of Raymond *et al* (1964) was followed for lipid estimation. Calorific value was estimated using a semi micro bomb calorimeter (Parr Instrument Company, USA).

The scheme for energy budget followed here is the slightly modified IBP formula (Petrusewicz and Macfadyen 1970) $C=P+R+F$; where C the food consumed, P the growth, R the weight loss due to metabolism and F the undigested food defecated including nitrogenous excretory products. Food consumed was estimated following the method of Mathavan and Pandian (1974). Assimilation was estimated by subtracting F from C; P was estimated by subtracting the initial dry weight of the larvae at the commencement of the experiments from the final dry weight of the same at the end of the experiment. Rates of feeding (Cr), assimilation (Ar), conversion or production (Pr) and metabolism (Mr) were calculated by dividing the respective amounts of weight by the product of mid-body weight (g) of the animal and duration (day) required for the completion of the life stage; the rates are expressed in terms of mg/g live insect/day. The gross conversion efficiency (K_1) expressed the percentage of food converted in relation to the food consumed, while net conversion efficiency (K_2) refers to the percentage of food converted in relation to assimilated food.

3. Results

Larvae restricted to feed 3 h/day survived for about 7 days and subsequently died. Restriction of feeding duration in other groups resulted in slow growth and extended larval duration. Larvae fed for 24 h/day required only 7 days to complete the feeding period whereas those restricted to feed 12 and 6 h/day required about 8 and 11 days, to pupate successfully (table 1). However, pupal period remained the same (8 days) in all the groups.

Table 1 presents the data on the mass budget of fifth instar larvae of the silkworm, fed on mulberry (*Morus alba*) at different restricted feeding durations. Food intake decreased from 3810 mg in the group fed *ad libitum* to 1900 mg, when the feeding duration was reduced to 6 h/day. Assimilation, conversion and metabolism increased with increasing feeding duration.

Rates of consumption, assimilation, conversion and metabolism showed steady increase, with the increase in the feeding duration (table 1). For instance, consumption rate was 156 mg/g/day in the group fed for 6 h/day; it increased to 339 mg/g/day in those fed *ad libitum*. However, net conversion efficiency of 34% in the 24 h feeding group has been elevated to 42% in those restricted to feed for 6 h/day (table 1).

Table 1. Effect of restriction of feeding duration on energy budget of *B. mori*

Parameters	Feeding duration			
	6 h	12 h	18 h	24 h
Instar duration (days)	11	8	7	7
Pupal duration (days)	8	8	8	8
Consumption (mg)	1900	2550	3730	3810
Assimilation (mg)	664	955	1396	1432
Conversion (mg)	277	318	442	488
Metabolism (mg)	387	637	954	944
Consumption.rate (mg/g live insect/day)	159	271	333	339
Assimilation rate (mg/g live insect/day)	56	101	125	128
Conversion rate (mg/g live insect/day)	23	34	39	43
Metabolic rate (mg/g live insect/day)	32	68	85	84
Net conversion efficiency (%)	42	33	52	34

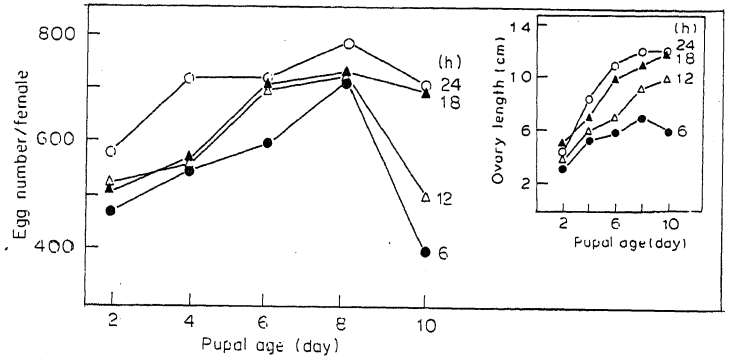

Figure 1. Egg number and ovary length of *B. mori* as functions of feeding duration and pupal age.

Restriction of feeding duration inflicted considerable change in the reproductive potential of *B. mori*. It is clear from figure 1 that with advancing age of pupa ovary length increased in all the groups; the ovary length doubled (12 cm) in the *ad libitum* group in comparison to the group fed for 6 h/day (6 cm). Corresponding to ovary length, egg number/female has also increased with increasing feeding duration (figure 1).

Data obtained for the egg weight of different age pupa and adult moths obtained from varying feeding schedules are represented in figure 2. The moths in the 24 h/day fed group almost doubled their ovary weight (990 mg) compared to the moths from the group fed for 6 h/day (480 mg).

Figure 3 represents the data on the changes of protein and fat content in the egg and the pupal tissues during the different days of pupal development. Percentage composition of protein in the egg increased with advancing pupal age in all the

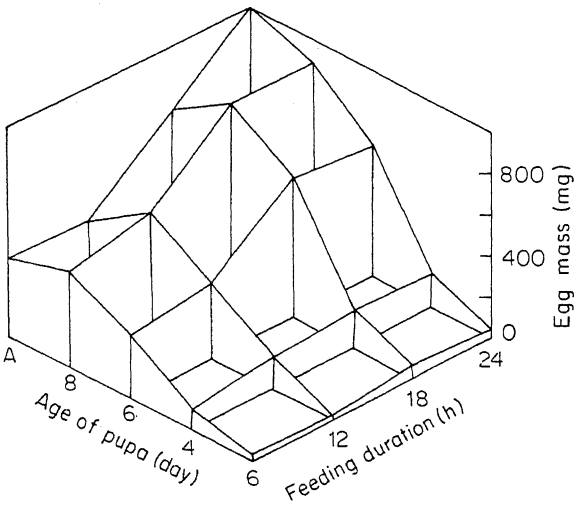

Figure 2. Ovary length and weight (wet) of *B. mori* as functions of feeding duration and pupal age.

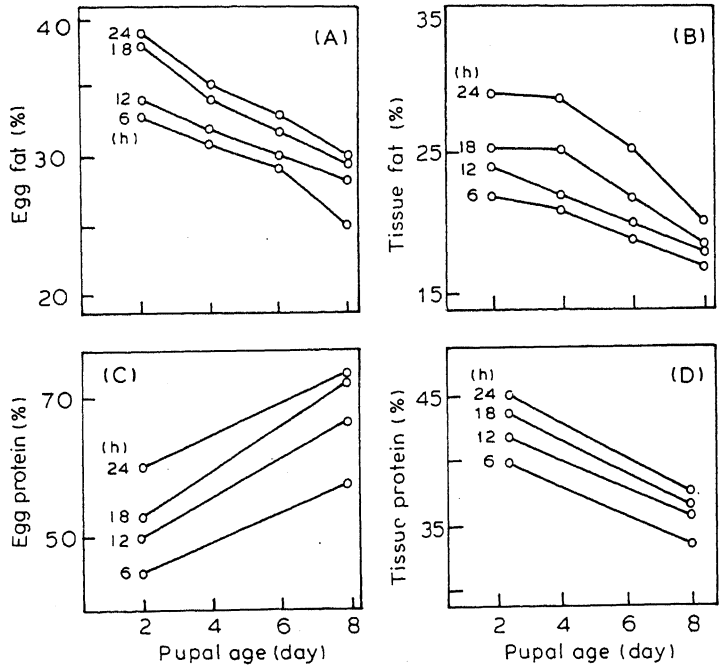

Figure 3. Changes in the percentage composition of protein and fat contents in the ovary and pupal tissues of *B. mori* during the course of pupal development.

experimental groups. However, protein content was considerably low in the egg obtained from 6 h/day fed group compared to the *ad libitum* fed group. The protein content in the pupal tissue was maximum during the early pupal age and it subsequently declined with the advancing age of pupa in all the experimental groups

(figures 3C and D). This observation shows that the protein from the pupal tissue is constantly transported to the egg in the process of vitellizing the egg with protein.

Egg and pupal tissues of *ad libitum* fed series contain considerably high concentration of fat than those obtained from restricted feeding schedules. However, fat content in the egg as well as pupal tissue steadily declined during the course of pupal development in all the experimental groups (figures 3 A and B).

While the energy content of egg increased with the advancing development, the energy content of the pupal tissue declined with the advancing pupal age. For instance, during the course of development, egg energy increased from about 5680 cal/g to about 6025 cal/g in the *ad libitum* fed group. The energy content of pupal tissue in the same feeding schedule declined from about 5950 cal/g to about 5500 cal/g (figures 4 A and B). Almost a similar trend is obtained in the changes of the energy content of egg and pupal tissues of other feeding schedules.

4. Discussion

The following adverse effects were obtained by restricting the feeding durations on *B. mori*: (i) Increase in the feeding larval period; (ii) decrease in the quantity of food consumed and converted as well as the rates of consumption and conversion; (iii) increase in the efficiency of conversion; and (iv) reduction in the reproductive potential.

In the present study, the larvae fed for 3 h/day suffered 100% mortality. These larvae extended their feeding period to more than the *ad libitum* fed group and still failed to spin. It may be true that the larvae require a critical feeding period of more than 3 h/day to complete the feeding larval period. The larvae restricted to feed for shorter durations extended their feeding period in order to obtain the maximum energy prior to pupation. Mathavan and Muthukrishnan (1976) reported that when restricted to feed for 3 or 6 h/day, *Danaus chrysippus* required 18 or 14 days to complete the final instar as against 6 days required by the larvae fed for 24 h/day.

Fed on decreased food abundance, the larvae compensate either by increasing in the feeding duration or by increase in the rate of feeding and/or efficiency of conversion. In the present study the *B. mori* larvae extended feeding duration at restricted feeding rations. The rates of feeding and conversion were low at the

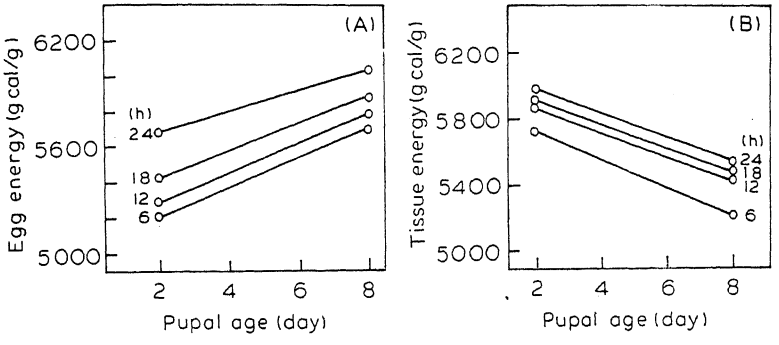

Figure 4. Energy content of egg (**A**) and body tissue (**B**) of *B. mori* as a function of feeding duration and pupal age.

restricted feeding group compared to the *ad libitum* fed group. Similar results were reported by Mathavan and Muthukrishnan (1976) and Christopher (1983) for lepidopterous larvae. Critical analysis of rates of feeding and conversion reported in the present study showed that the *B. mori* larvae compensate the restricted feeding duration by increasing consumption rate over the limit expected of it in proportion to the feeding duration. The feeding duration in the 6 h fed group was 4 times less than *ad libitum* fed group. However, the reduction in the consumption rate and conversion rate only two times less in the 6 h fed group than the *ad libitum* fed group. Further, *B. mori* larvae enhanced the conversion efficiency at the restricted feeding duration than the *ad libitum* fed group. Similarly, Muthukrishnan and Delvi (1974) reported that the grasshopper, *Poecilocerus pictus* partially compensated the diminishing ration by increasing the conversion efficiency. Thus the *B. mori* larvae try to compensate the stress of restricted feeding duration by increasing rates of feeding, conversion and efficiency.

Slansky and Scriber (1985) in the recent review on food consumption and utilization stated that considering reproduction, the physiological response of an insect for a decreased food abundance will be delayed or decreased reproduction. The oogenesis and oviposition, i.e. timing and rate of reproduction, number and quality of eggs depend on (i) the nutrient accumulated during the feeding larval period; (ii) the quantity and quality of the adult food; and (iii) the amount of nutrient allocation by the female for each egg (Engelmann 1970; DeWilde and DeLoof 1973b; Bell and Bohm 1975; Slansky 1980a, b; 1982; 1985). In insects in which the adult does not feed, reproduction depends on the success of the female attaining the ideal final body weight (Engelmann 1970; Hinton 1981). In the case of *B. mori*, the whole process of egg development is completed in the pupal period itself and on emergence the adults do not feed and they concentrate on depositing the eggs. Hence, the fecundity of *B. mori* depends on the amount of energy stored during larval period and on the attainment of adult weight. The amount of food allocated for egg production in *B. mori* fed for different feeding durations was calculated. Restricted food availability results on the reduced fecundity of *B. mori*. However, when related to the percentage of assimilated energy allocated for egg production, it is almost the same in all the feeding groups, i.e. they allocated about 15–19% of the assimilated energy for egg production. The data reported by Hiratsuka (1920) on the allocation of assimilated energy for egg was 12·5%. In general, lepidopterans allocate about 10–20% of total assimilated energy for egg production (Slansky and Scriber 1985). Amount of pupal energy allocated for egg production was about 55% in the 6 h/day fed group (figure 5). It amounted to 67% in those fed for 24 h/day. Energy allocation for egg by the pupae obtained from 12 or 18 h/day fed group did not vary significantly from that of 24 h fed group. In other words, the pupae from the 12/18/24 h fed group allocated energy for egg production almost with an equal efficiency.

Both in the egg and the pupal tissue, percentage composition of fat steadily decreased with an advancing pupal age in all the feeding regimes. It is because fat served as a major source of energy for metabolism during pupal development (see also Crescitelli 1935; Klekowski *et al* 1967). It has been shown that during vitellogenesis, considerable amount of lipoproteins are deposited in the egg which originate from fat reserves of pupal tissue (Chino *et al* 1977; Lubzens *et al* 1981). However, the protein and energy content of egg constantly increased with advancing age and feeding regimes. More than 80% of the body protein was deposited into the

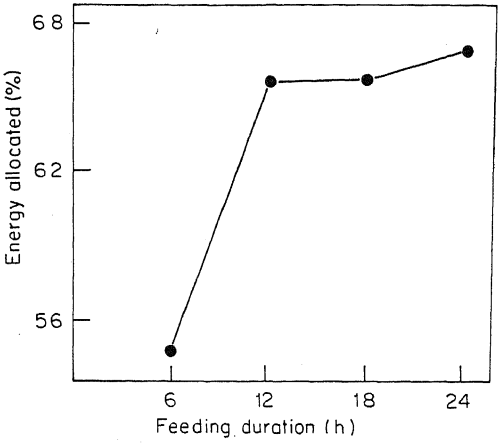

Figure 5. Percentage of energy allocation for egg production in *B. mori* reared on different feeding durations.

egg during vitellogenesis in those fed *ad libitum*. The incorporation of body protein into the egg was only about 40% in those fed for 6 h/day. It is evident from the results that individuals in the restricted feeding group accumulated less protein in the egg than the maximum fed group since the protein available for incorporation is less in the former than the later feeding group.

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Influence of azadirachtin on insect nutrition and reproduction

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Abstract. Neem extracts and pure azadirachtin influence the behaviour and physiology of large number of insects. These compounds affect the sensory receptors, thereby inhibit food intake. This anti-feedant property is exploited by using neem extracts and azadirachtin for plant protection. The gut motility and movement of food in the digestive tube are regulated by azadirachtin. *Holotrichia serrata*, the beetle of the 'white grub' feeds on neem leaves and no ill-effect of azadirachtin is reported.

Azadirachtin and neem extracts regulate the reproductive functions of insects through their endocrine system. Morphogenesis, ovarian development, fecundity, egg viability and moulting are adversely affected by azadirachtin. Radioimmunoassays show that the ecdysteroid level falls drastically on azadirachtin treatment. Juvenile hormone synthesis also is inhibited in *Locusta* on administration of azadirachtin. In *Oncopeltus fasciatus*, high doses of azadirachtin inhibit adult development resulting in permanent larvae which show oocyte differentiation with yolk incorporation equivalent to day 4 of adult development.

Keywords. Azadirachtin; neem; nutrition; reproduction.

1. Introduction

Azadirachtin, a triterpenoid isolated from the Indian neem tree, *Azadirachta indica* A Juss, like many other plant products has varied effects on the behaviour and physiology of insects. Crude neem extracts and pure compounds like azadirachtin, salanin, meliantroil and toosendanin derived from the seed kernel were used to study their insecticidal properties. The biocidal action of neem products was known for centuries in India and leaves and seeds were used by the farmers to protect stored products from insect attack. More recently, neem extracts and azadirachtin are extensively used to study their insect repellant, anti-feedant and growth regulator properties.

Azadirachtin was isolated from neem seed and its chemical composition elucidated by Butterworth and Morgan (1971) and Zanno *et al* (1975). Large number of investigators have studied the effects of whole and partially purified extracts, azadirachtin and other pure fractions on various insect species (Ruscoe 1972; Rembold *et al* 1982; Steffens and Schmutterer 1982; Garcia *et al* 1984; Dorn *et al* 1986; Kalyanasundaram and Babu 1982; Ladd *et al* 1984; Prabhaker *et al* 1986; Meisner *et al* 1985; Zebitz 1984; Mordue *et al* 1985; Gujar and Mehrotra 1983; Hellpap and Zebitz 1986; Schmutterer 1985).

Extensive research during the past decade has brought to light, the insect repellant, anti-feedant, growth disruptive and reproductive inhibitory properties of neem seed extract and pure azadirachtin. The cumulative effects of the neem extract on insects have led to the emergence of a new potential insecticide. Over 150 species of insects show feeding deterrence and 30 species ovipositional inhibition and over 70, growth and reproductive inhibition, when they or the host plants are treated with azadirachtin or alcoholic/aqueous extracts of neem.

This paper deals with the influence of azadirachtin and neem extracts of various grades of purification on insect nutrition and reproduction.

1.1 *Influence on nutrition*

Azadirachtin affects the total sensory activity of receptors of maxilla, thereby inhibiting food intake in species like *Heliothis* spp. and *Spodoptera littoralis* (Simmonds and Blaney 1983; cf. Mehrotra and Gujar 1986; Meisner *et al* 1983). Azadirachtin and a commercial preparation of neem seed extract (margosan-O) and methanolic and aqueous extracts of the leaves and seeds are effective anti-feedant on various insect groups (Attri 1975; Saradamma *et al* 1977; Adler and Uebel 1984, 1985; Mariappan and Saxena 1983; Kareem *et al* 1974; Arnason *et al* 1985). A comprehensive list of insect species thus affected is given by Mehrotra and Gujar (1986). The cotton flea beetle, *Podagrica* spp. and the leaf roller, *Sylepta derogata* attacking the okra plants (*Hibiscus esculentus*) do not feed on the leaves when methanolic extracts of neem seed and leaf are sprayed at weekly intervals (Adhikary 1984). Redfern *et al* (1984) also have found that the ethanolic extract from fresh neem seeds at 0·2 and 0·4% aqueous solutions retained the anti-feedant potency for a period of 21 days. However, pure azadirachtin rapidly looses 50% of its anti-feedant property within 7 days of exposure to sun light. By 16 days complete potency will be lost (Stokes and Redfern 1982). The potency could be retained for longer period by mixing azadirachtin with plant oils. Mixtures of seed oils of *Anona squamosa* and *Azadirachta indica* in different proportions show high potencies in controlling *Nephotettix* (Mariappan and Saxena 1984).

Neem extracts, ground seed and deoiled cake are used to protect stored products from grain pests such as *Rhizopertha dominica*, *Tribolium castaneum* and *Sitophilus granarius* (Jilani and Helen 1983; Ambika and Mohandas 1982; Pereria and Wohlgemuth 1982; Malik and Mujtaba Naqvi 1984; Singh and Singh 1985). Dry ground neem seed mixed with cowpea and maize prevented *Callosobruchus* and *Sitophilus zeamais* from feeding (Ivbijaro 1983a, b).

Neem seed kernel suspension at 0·5, 0·75 and 1% protect the tobacco crop from the attack of *Spodoptera litura* (Joshi *et al* 1982, 1984; Meisner *et al* 1983). Similarly, aqueous suspension of both seed and leaf extracts prevent the diamond back moth, *Plutella xylostella* from feeding the cabbage heads (Adhikary 1985). Fagoonee and Lange (1981) have reported that a concentration as low as 0·001% methanolic extract as highly toxic to *Crocidolomia binotalis*. Neem oil and deoiled cakes are effectively used against the brown hopper, *Nilaparvata lugens* and the food intake by the nymphs was very much reduced (Saxena *et al* 1984; Krishnaiah and Kalode 1985; Chiu Shin foon *et al* 1983).

Azadirachtin A and B and 7-acetyl azadirachtin, when fed to *Rhodnius prolixus* through blood at a concentration of 25–30 μg/ml acted as anti-feedant. This effect could be reversed by orally administering ATP, a phagostimulant (Garcia *et al* 1984; Garcia and Rembold 1984). In the cucumber beetle, *Acalymma vittatum* and *Diabrotica undecimpunctata*, both azadirachtin and salanin act as anti-feedants (Reed *et al* 1982). Similar feeding inhibition is found in *Phyllotreta striolata*, *Dysdercus koenigii*, *Spodoptera litura*, *Dissosteira carolina*, *Ostrina nubilalis* and 6 species of

cockroaches (Meisner and Mitchell 1982; Arnason *et al* 1985; Adler and Uebel 1984, 1985; Koul 1984, 1985).

Azadirachtin has significant influence on the passage of food through the gut by affecting the gut motility in *Locusta migratoria migratorioides* (Mordue *et al* 1985).

It is evident from the earlier studies that neem products and pure azadirachtin elicit anti-feedant activity. However, in *Holotrichia serrata*, the adult beetles feed on neem leaves. These beetles emerge after a prolonged pre-emergent adult stage. An active feeding is followed by the maturation of oocytes in this insect (G K Karnavar, unpublished). No information is available on the role of azadirachtin present in the diet on the physiology of this insect.

2. Influence on reproduction

2.1 *Through endocrine system*

Neem extracts and azadirachtin have physiological action on the endocrine system of insects affecting the moult cycle, ovarian development, fecundity, oviposition and egg viability (Schluter *et al* 1985; Redfern *et al* 1981; Ascher and Gsell 1981; Prabhaker *et al* 1986; Mordue *et al* 1985; Dorn *et al* 1986). A single injection of 10 μg azadirachtin resulted in sterilizing effect on *Locusta migratoria migratorioides*. The maturation of the terminal oocytes was arrested and there was no sign of oviposition in such insects. Radioimmunoassay of the hemolymph showed a low level of ecdysteroid (Rembold and Sieber 1981; Sieber and Rembold 1983). In the fifth instar nymphs of *L. migratoria*, Mordue *et al* (1986) reported that ecdysteroid level drastically dropped on injection of azadirachtin, consequently the air swallowing capacity was very much impaired and moulting inhibited.

The hemolymph ecdysone content of the larvae of *Ostrinia furnacalis* is very low and brain, corpora cardiaca and corpora allata show histopathological changes when the insects are treated with 20 ppm azadirachtin (Shin foon Chiu *et al* 1985). Anti-ecdysteroid activity of neem extract is also reported in the sweet potato white fly, *Bemisia tabaci* and *Oncopeltus fasciatus* (Coudriet *et al* 1985; Redfern *et al* 1982). In these two, treated larvae failed to pupate and mortality was very high.

Rembold *et al* (1984) have reported a complete inhibition of juvenile hormone synthesis following the injection of azadirachtin in *Locusta*. Similar effect is found in *Manduca* larvae also (Schluter *et al* 1985).

Based on the effects of azadirachtin on moulting, the doses are classified as low, medium and high by Dorn *et al* (1986). Low doses (0·00098 to 0·03123 μg/larva) of azadirachtin did not prevent adult formation but ecdysis was retarded in *Oncopeltus fasciatus*. While medium doses (0·0625 to 0·25 μg/larva) suppressed ecdysis completely and the larvae remained permanent. At high doses, both ecdysis and apolysis are blocked. Garcia and Rembold (1984) have shown that the ecdysis inhibition in *Rhodnius prolixus* was linearly related to the log of azadirachtin dose. Ecdysis inhibition by azadirachtin is due to an interference with the effects which are under ecdysteroid control. Azadirachtin may induce ecdysis inhibition by blocking ecdysone production by the prothoracic gland (Sieber and Rembold 1983). In *Musca autumnalis*, azadirachtin inhibits both adult development and reproduction (Ibrahim and Hayes 1984).

2.2 *Growth disruption*

Crude methanolic extract of neem and pure azadirachtin cause morphogenetic effects in *Disdercus* (Koul 1984). *Mythemna seperata* fifth or sixth instar larvae fed on leaves treated with partially purified neem extract showed high degree of mortality and the survived larvae developed into pre-pupae (Schmutterer *et al* 1983). Azadirachtin when fed with artificial food at the rate of 20 ppm to the third and fourth instar larvae of *Ostrinia furnacalis*, the larval period was prolonged. This inhibition is due to low β-ecdysone level (Shin foon Chiu *et al* 1985).

The growth disrupting property of azadirachtin on *Epilachna varivestis* show that a dose less than 0·3 μg could inhibit metamorphosis (Schluter 1985) and 5–10 ppm for 48 h sufficient for growth inhibition in *Spodoptera litura* (Koul 1985). Methanolic extract of neem kernel with tropital increases the growth disrupting effect in the same insect (Lange and Schmutterer 1982). 100% mortality in *Leptinotarsa decemlineata*, *Pieris brassicae* and *Memestra brassicae* was found at a level of 100 mg/kg (Feuerhake and Schmutterer 1982).

2.3 *Fecundity and egg viability*

Egg hatchability is affected in *Musca autumnalis* when treated with azadirachtin (Ibrahim and Hayes 1984). In the case of the stored product insects also, the productivity is effectively reduced by neem seed extract (Pereira and Wohlgemuth 1982; Pereira 1983; Ivbijaro 1983a). The fecundity rate of the carmine spider mite, *Tetranychus cinnabarinus* is affected by the neem seed extract (Mansour and Ascher 1983). Growth retardation and impairment of fecundity are reported in the green rice hopper, *Nephotettix virescens* (Von Hyde *et al* 1985). *Schistocerca gregaria* treated with azadirachtin (2 μg/g) showed a complete inhibition of the development of the reproductive system. Moreover, hemolymph proteins also decreased qualitatively and quantitatively (Subramonyam and Rao 1986).

The repellant effect of neem extract and azadirachtin results in the feeding deterrence in some insects and anti-oviposition effect in others. In *Liriomyza sativae*, aqueous solutions of neem seed extract applied to leaves prevented oviposition (Webb *et al* 1983). Larval mortality after hatching was 100% in such treated plants. Similar reduction in egg number and viability are reported in *Corcyra cephalonica* after exposure to neem oil (Pathak and Krishna 1985).

2.4 *Oocyte development in permanent larvae*

A significant observation of the development of oocytes in the permanent larvae of *Oncopeltus fasciatus* induced by the administration of high doses of azadirachtin is reported by Dorn *et al* (1986). At a dose of 0·5 μg/larva, 45% of the female larvae show globular yolk incorporation in the oocyte, which normally starts at day 4 in the adult females. No evidence regarding the action of azadirachtin on the endocrine system responsible for oocyte maturation is available in the above studies except the comparison of the time span between ecdysteroid peak and onset of egg maturation in normal and treated individuals.

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Host dependency among haematophagous insects: A case study on flea-host association

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Abstract. Obligatory haematophagy is the end result of long standing interspecific associations. Present day specificities to host, blood meal and physiological stage of the host are all offshoots of the primitive interspecific associations. The cause/effect relationship of these dependencies and specificities are probably based on the route through which haematophagy evolved in different groups of insects.

In the present analysis, flea-host association is taken into consideration. It is possible to find an array of host relationships ranging from promiscuous and catholic host associations to strict ones. In general 3 categories may be recognized. In fleas like *Xenopsylla cheopis* a utilizable protein in an optimum concentration gives the necessary stimuli for maturation (Cheopis-type), while in a second group the stimuli is provided by certain circulating hormones of the host (Cuniculi-type) as seen in rabbit flea *Spilopsyllus cuniculi* and in a third group (Monositus-type) a priming period characterized by tissue fluid feeding and neosomy is necessary before whole blood diet can stimulate maturation as exemplified by *Tunga* spp.

It appears that vertebrate associations of Siphonaptera initiated as adaptations to the nest microhabitat and haematophagy and adaptations to physical/chemical factors of epidermal habitat being subsequent developments.

Keywords. Haematophagy; flea-host associations; nest microhabitat; epidermal habitat.

1. Introduction

Obligatory haematophagy is the end result of long standing interspecific associations. Such associations lead to metabolic dependency and/or dependency to physical and/or chemical environmental factors relating to the breeding place of the host and/or epidermal habitat offered by the vertebrate host.

According to Smyth (1962) metabolic dependency with regard to endoparasites may be evident in one or more of the following: (i) developmental stimuli; (ii) nutritional material; (iii) digestive enzymes and (iv) control of maturation. Blood feeding triggers not only the development of ovary in the adult but also influences moulting in exopterygote haematophages. This influence is negotiated through the hormones secreted as a result of either the mechanical stimuli from proprioceptors of gut/abdomen or chemical stimuli from diffusible substances or digestion products from blood meal. In all haematophagous insects blood meal supplies nutritional materials necessary for reproductive and house keeping metabolic functions. Nematoceran and brachyceran Diptera, however, supplement their blood meal with phytoexudates or nectar and the carbohydrates derived from this source are mainly used for flight and routine metabolic functions. Fritz (1983) mentioned that as dextrose was present in low concentration in blood, acquisition of the necessary sugars for haematophagous insects like streblids which feed only on blood, was by frequent blood feeding. This requires experimental verification. Clements (1963), Engelmann (1970), Nelson *et al* (1975), Prasad (1987) and others deal with nutrition

and reproduction of haematophagous insects. Ectoparasites are not dependent on the host for supply of digestive enzymes, because they are adequately equipped with the necessary enzymes to digest the blood meal. According to Waage (1979) evolution of many dipteran/vertebrate associations have come about through the advantage gained by those species which are able to digest their host's blood cell proteins and use it for longevity and reproduction. Depending on the route through which haematophagy evolved, the group may already possess the necessary protease (pre-adapted) or may evolve the digestive capacity more gradually (Waage 1979). But it is important to remember that some of the dietary proteins control the rate of midgut protease synthesis and the quantity secreted. Maturation is a host dependent feature of haematophagous exo- and endopterygotes. Except in a few cases of autogenous egg production, stimulation of ovaries from resting stage at emergence depends on blood intake by the insect. Similarly though not spermatogenesis (in certain ticks even spermatogenesis is under the control of blood feeding) mating competency in male is induced only after blood feed.

These points clearly indicate that haematophagous arthropods are metabolically dependent on the host for several functions. Waage (1979) suggested that the nature and intimacy of an ectoparasite/host relationship will depend on the sequence in which regular physical associations (habitat preference, host seeking behaviour and morphological adaptations promoting capacity to feed on the host, physiological capacity to digest host substances and behaviour associated with initiation and termination of feeding) occur.

Preferences and adaptations to microenvironment of the epidermal habitat is an important component of host specificity. The type of integument of the host (feather vs hair), the temperature conditions of the epidermal habitat, thickness and texture of skin, the extensiveness of the peripheral circulation etc can be very decisive factors in host preferences. Among many insect species non-nutritional chemicals play primary role in determining host selection and suitability (Slansky and Scriber 1985). Subsequent to Hosoi's (1958) discovery of the importance of adenine nucleotides as phagostimulants for the mosquitoes *Culex pipiens* several studies have shown that many unrelated groups of haematophagous insects show adaptation to same phagostimulatory chemicals (convergent evolutionary trend) as well as marked diversity in mechanism of blood recognition (Galun 1986). Galun and Rice (1971) found that the source of adenine nucleotide at least for *Aedes aegypti* and *Glossina austeni* is blood platelets. They suggested that quantitative variations of platelet ATP of different mammals could contribute to differences in host preferences of haematophagous insects. The diversity which Galun (1986) speaks about relates to those which recognize blood through the properties of plasma, yet others through some cellular fractions and a third group somewhat intermediate between these.

Having thus outlined some generalities regarding host-haematophagous arthropod associations, a more specific case of flea-host association and specificities may be examined.

2. Flea-host association

The maximum flea representatives parasitize rodents and insectivores (table 1). Undoubtedly the lifestyle of the host (foraging, breeding and nest/burrow

Table 1. Distribution of flea families on mammals and birds.

Host record	Flea families	Host record	Flea families
Monotremata	Pulicidae, Stephanocircidae	Rodentia	Ceratophyllidae,
Marsupialia	Hystrichopsyllidae,		Coptopsyllidae,
	Pygiopsyllidae		Hypsophthalmidae,
Insectivora	Ceratophyllidae,		Hystrichopsyllidae,
	Chimaeropsyllidae,		Leptopsyllidae,
	Hystrichopsyllidae,		Macropsyllidae,
	Leptopsyllidae,		Pulicidae,
	Pulicidae		Pygiopsyllidae,
Chiroptera	Ischnopsyllidae		Rhopalopsyllidae,
Edentata	Malacopsyllidae,		Stephanocircidae,
	Tungidae		Tungidae
Lagomorpha	Hystrichopsyllidae,	Carnivora	Ancistropsyllidae,
	Leptopsyllidae,		Ceratophyllidae,
	Pulicidae		Malacopsyllidae,
			Pulicidae
			Vermipsyllidae
		Ungulata	
		Perissodactyla	Vermipsyllidae
		Artiodactyla	Ancistropsyllidae,
			Pulicidae,
			Vermipsyllidae
		Aves	Pulicidae,
			Rhopalopsyllidae,
			Tungidae

construction behaviours) influences considerably this differential rate of parasitization. Both these groups of hosts live in some sort of nests made in crevices or burrows. Even among bird fleas, most of these are found associated with species of birds which breed on ground and in banks or use mud freely in the construction of their nests (Rothschild and Clay 1952). Those which live on hosts without regular nesting sites have hypertrophied anchoring devices and would be highly promiscuous (Hopkins 1957; Traub 1980). Speaking of host association of fleas Holland (1964) stated that some species of fleas will breed on only a single species or genus of animal (as all *Amphalius*, *Geusibia* and *Ctenophyllus* spp. on *Ochotona* spp.) others may live on a more-or-less related group of hosts (as *Chaetopsylla setosa* Rothschild on many large carnivora); others again apparently breed indiscriminately on hosts sharing a common habitat (as *Ctenophthalmus pseudagyrtes* Baker) on shrews, moles and microtine and cricetine rodents or *Dasypsyllus gallinulae perpinnatus* Baker on many species of Passeriformes and other birds. Based on the degree of closeness of fleas to their hosts, they may be broadly classified into nest ectoparasites (nest-burrow blood suckers) and host ectoparasites. While the former are found most of the time off the host (certain species of *Coptopsylla* and *Ceratophyllus*) the latter either remain attached to the body of the host for the whole life time (slow feeding intradermal parasites) such as *Tunga* and *Echidnophaga* or spend more time on the host in a free state moving about among the fur like *Ceratophyllus*, *Leptopsyllus*, *Xenopsylla* and others which are also fast (rapid) feeders taking several blood meals (Balashov 1984; Nelson *et al* 1977). The distribution and

host specificities of the nest fleas are controlled by the microenvironmental conditions prevailing in the nest of the host animal. To a certain extent this would be true of the other groups also. Hopkins (1957) qualified fleas as nest specific rather than host specific, because the larvae are free-living and are considerably influenced by the nesting conditions of the host. There are several instances to show that nesting conditions of hosts influence the distribution and host preference/specificity of fleas. Sharif's (1948) studies in the Deccan Plateau showed that rat fleas *Xenopsylla cheopis* and *X. brasiliensis* were predominantly associated with domestic rats, whereas *X. astia* with field rats like *Bandicota* and *Tatera*. He suggested larval nutrition to be the most important factor in this pattern of distribution. According to him the larvae of *X. astia* were dependent on the presence of vitamin B in the diet whereas the other two species could grow even in its absence. In nature the profuse growth of fungi in the burrows of *Bandicota* and *Tatera* provide the necessary B-vitamins whereas the comparatively dry nests of domestic rats do not encourage fungal growth and so are not suitable for *X. astia*. While Sharif's nutritional hypothesis helps to give possible explanation for the preponderance of *X. astia* on semi domestic and wild rodents like *B. malabarica* (=*B. indica*) and *T. indica* in the places of his study, it does not explain the comparative absence or rarity of *X. cheopis* from the nests of these hosts. In spite of the actual interchange of fleas between semi domestic and domestic rats (Annual Reports of the Haffkine Institute; Prasad 1966) burrow faunistic studies of field rodents show the absence or rarity of *X. cheopis* (Joshee 1961; Kamath 1961). It would be unwise to say that *X. cheopis* cannot breed in a nest which contains nutritionally rich litter. It could be that the excess of ground moisture is injurious to *X. cheopis* larvae. *X. cheopis* is known to have a prediliction for comparatively dry situations (Pollitzer 1954). Another interesting instance is the distribution of *X. nubica* which is specific in northern Uganda to Gerbillinae of two genera. The preferred species of Gerbil is common in southern Uganda but here the flea is absent presumably because the gerbil burrows here are too damp. Burrow conditions similarly appear to control the distribution of *X. cheopis* which in East Africa is restricted to *Rattus rattus* in the southern areas, but occurs freely on a wide variety of field rats in the northern areas possibly because the burrows of field rats in northern area offer a similar drier condition as those of *R. rattus* in the southern areas. Moisture conditions of the nesting sites seem to affect the distribution of bird fleas also. The hen flea *Ceratophyllus gallinae* is found more frequently than any other fleas in the nest of sparrows, starlings, sparrow-hawks, swallows etc where comparatively dry atmosphere prevail. The moorhen flea *Dasypsyllus gallinulae* prefer nests with damp conditions such as those of robin, warblers, finches, blackbirds etc. The duckflea *C. garei* on the other hand can survive in wet swampy situations which prove fatal to the other two species of fleas and this is the only flea found in the nest of ducks, geese and certain waders and sea birds. That the distribution and host preferences are not guided by the nesting conditions alone (at least in some cases) is interestingly illustrated by the distribution of these 3 bird fleas in the nests of pheasants and partridges. Nesting habits of these birds are similar and they often use each others nest. Yet *C. gallinae*, *D. gallinulae* and *C. garei* are found in the nest of pheasant, but *C. garei* is the only flea in partridge nest.

There is very little information on the role of host odour influencing host association of fleas. The existing information are varied. The studies of Benton *et al* (1959) indicate preference of *Sylvilagus floridanus*, the natural host, by the flea

Cediopsylla simplex over an opossum *Didelphis marsupialis* in twin host choice experiments. Bates (1962) negated the possibility of olfaction as an important factor in host finding by bird fleas. Shulov and Noar (1964) found the rat fleas *X. cheopis* making a clear distinction between hosts, preferring white rat to 3 other species of murids *Meriones tristrami*, *Acomys dimidiatus* and *Microtus guentheri*. Humphries (1968) found olfaction playing no role in host location by the hen flea *Ceratophyllus gallinae* while Vaughan and Mead Briggs (1970) found that rabbit flea *Spilopsyllus cuniculi* was strongly attracted to the urine of the host, but showed no host specificity. Benton *et al* (1959) found *Ctenophthalmus pseudogyrtes* unable to recognise its natural host the chipmunk *Tamias striatus* from distances exceeding two inches. A static air olfactometer showed that the rat fleas *X. cheopis* and *X. astia* are neither able to distinguish the presence of a host nor are they able to discriminate between *R. rattus*, *R. norvegicus* and *Mus musculus* even at short distances of 6 cm (K Jaya and R S Prasad, unpublished results). In these cases the host choice take effect after reaching it as shown by Sgonina (1939, cited by Humphries 1968) for certain mammal fleas. This is strengthened by the fact that there is an interruption of regular feeding and breeding if the antennae of the female fleas are blocked before releasing them on live host. Though a small percentage of female *X. astia* whose antennae were painted with nail polish and the maxillary palps ablated before releasing on the host, were blood fed, none laid eggs (R S Prasad, unpublished results). Whether some host-borne chemical signal is involved or not is not known. It is significant to remember that flea transfer between unrelated hosts such as prey to predator, dog/cat flea to human beings etc (Hunter *et al* 1979) are probably indications of want of ability to distinguish interspecific differences in host-borne cues. However, an air-borne kairomone emanating from the new born young rabbit which is also present in the urine is involved in boosting copulation and egg production in *S. cuniculi* (Rothschild and Ford 1969, 1973).

At least in the case of rat fleas it has been shown that defensive behaviour of the host influences not only the establishment of flea ectoparasites on the host but also their breeding potentials (Lila Chandy and Prasad 1987). For example mortality of adult fleas was high and fecundity rate low on white mouse, *M. musculus* albino, whose grooming activities are more intense than white rat *R. norvegicus*. Variations in grooming activities associated with age and sex of the host appeared to be important in intraspecific differences noticed in mortality (higher on adult and female rats compared to young and male rats respectively) and fecundity rates (higher on young and male rats compared to adult and female rats respectively).

Since all the species of adult fleas have only blood as their food, these have to derive the nutrients necessary for meeting day to day metabolism as well as reproductive functions from blood itself. Frequent feeding is a characteristic of fleas except those such as *Tunga* spp. which are slow and continuous feeders. The question as to how far do variations in the blood components affect host suitability and specificity is difficult to answer as there is very little information on the nutritional compatibility of the blood from different hosts. From early 1900 there are indications of interspecific variations in host suitability. Bacot (1914) noticed that only when fed twice would *X. cheopis* lay eggs on human beings, even then the fecundity was very poor. Strickland (1914) found *Ceratophyllus* (= *Nosopsyllus*) *fasciatus* fed on mouse, rabbit or man did not lay eggs, but if the same lot was allowed to feed on rat, egg laying resulted shortly

after feeding. Haas (1965) studied the comparative suitability of 4 murine rodents to fleas *X. cheopis* and *X. vexabilis* and showed that wild *M. musculus* figured as the most unsuitable host for both the species of *Xenopsylla* whereas *R. exulans* appeared to be the best host for *X. vexabilis* and *R. norvegicus* for *X. cheopis*. Prasad (1969) based on comparative fecundity studies of *X. cheopis* concluded that even though flea mortality was high on *R. rattus* and *M. musculus* (albino) and was low on *Bandicota bengalensis*, the former two hosts proved most suitable for *X. cheopis*. Samarina *et al* (1968) showed that fertility value of *Ceratophyllus consimilis* was highest when fed on hamster than on rat or mouse. This was found to be true for *C.* (=*N.*) *fasciatus* and *X. cheopis*. Bibikova (1965) found that feeding on unspecific host usually takes much longer and blood of usual host digests faster. The results of direct feeding of fleas need not necessarily reflect nutritional influences because there would be several non-nutritional parameters interfering feeding and quantity of blood ingested. The pupal production of human strain of *Pulex irritans* fed directly on man or artificially on citrated human blood was much higher than when fed on dog/citrated dog blood. Fox strain of *P. irritans* on the contrary did not reproduce when fed either directly on man or artificially on citrated human blood. The pupal production of the same strain was high when fed directly on dog, but did not reproduce when fed artificially on citrated blood of dog (Hudson and Prince 1958). Experiments involving artificial feeding of rat fleas *X. cheopis* and *X. astia* on heparinised blood of frog, chick, white rat, house rat, white mouse and man through the skin of a young white rat (the usual host for laboratory rearing of these fleas) showed that all the blood samples could induce yolk deposition, but a significantly higher percentage of *X. astia* fed on *R. rattus* blood showed yolk deposition while a significantly lower percentage of *X. cheopis* showed yolk deposition with chick blood. Similar experiments to assess egg laying potential when fed on heparinised blood of frog, hen, white rat, house rat, gerbil and man showed no significant difference in egg production attributable to the interspecific variations in blood sources, whereas with regard to *X. cheopis* no definite conclusions could be drawn due to poor gorging and erratic egg laying. However, egg laying started on the 9th day on blood of house rat whereas on blood from other sources egg laying started only around 13–16 days after the first blood meal. No such marked difference could be noticed with *X. astia* where egg laying started at the earliest by the 4th day and latest by the 7th day. These blood samples were offered through the skin of a young white rat, a host with which these fleas are familiar in the laboratory as white rat is used as host for laboratory rearing of these fleas (Kamala Bai and Prasad 1976, 1981). Feeding these fleas artificially on blood fractions of white rat showed that blood cell fraction triggered yolk deposition but plasma alone did not. The factor associated with this nutritional inadequacy of blood plasma was shown to be the protein concentration because when plasma was fortefied with rabbit albumin, yolk deposition was initiated (Kamala Bai and Prasad 1976). Subsequent studies on artificial diet showed that both quality (fleas were not able to utilize bovine albumin in place of bovine haemoglobin for yolk synthesis) and quantity (twice fed fleas laid more eggs than those fed once artificially) of protein are important and that a diet balanced with salts, sugars, cholesterol and B-vitamins in addition to protein and a phagostimulant (ATP) was necessary for vitellogenesis. However, B-vitamins appear to be dispensible for *X. astia* (Kamala Bai and Prasad 1979, 1981). It then follows that the amino acid composition of blood proteins is an important attribute in

conferring nutritional adequacy of the blood meal. However, attempts to feed these fleas on artificial diets in which protein is replaced with amino acids failed.

Synchronization of breeding activities of the host and its flea ectoparasite has been demonstrated in the case of rabbit fleas *S. cuniculi*. Before the breeding season commenced female rabbits carried an extra load of fleas (Allan 1956). Following the observation of Allan (1956), Rothschild and Ford (1964a) discovered that the rabbit flea's ovaries can mature only on a doe rabbit during the last 10 days of its pregnancy or on a new born baby rabbit during the first 5 or 6 days of life. Their further work revealed that maturation was probably initiated and maintained only when corticosteroids circulating in the blood reached a relatively high level. Ovarian maturation induced as a result of direct spraying of hydrocortisone or corticosterone on the flea revealed a direct action of these hormones on the flea (Rothschild and Ford 1964b). Lutinizing hormone and progestins were found to induce ovarian regression. Growth hormone secreted by anterior pituitary constituted one of the major copulation factors (Rothschild and Ford 1966) which accounted for the fact that copulation of rabbit fleas took place only on nestling rabbits. Maturation of male rabbit flea *S. cuniculi* (defined as the stage of development at which sperm can be successfully transferred to the female spermatheca) is also under the influence of host's hormones (Rothschild *et al* 1970). Rothschild and Ford (1969, 1973) showed that in addition to the hormones described above there are certain pheromone-like nestling factor (kairomone) from the new-born young rabbits which speed up maturation and copulation and enhance sperm transfer. The rabbit flea *S. cuniculi* is not an isolated case of hormonal dependency. Rothschild and Ford (1972) showed that the breeding of the rabbit flea *Cediopsylla simplex* is similarly synchronized with the breeding cycle of the host. According to Rothschild (1965) *Ischnopsyllus*, the bat flea, could be another case where an ectoparasite respond to the sexual cycle of the host. These fleas crowd on female bats before they leave their winter quarters for their summer breeding roosts.

One of the most fascinating flea-host association is that of the so-called sticktight and chigoe fleas. The former is exemplified by the notorious pest of poultry *Echidnophaga gallinacea* while the latter by *Tunga* spp. In both the cases the females are permanent parasites on the body of their respective hosts. Continuous feeding, mating and egg laying are accomplished while firmly anchored on the body of the host. However, there are some major differences in feeding of these fleas. *Echidnophaga* feeds on whole blood soon after attachment whereas the work of Lavoipierre *et al* (1979a) show that *T. monositus* pass through 3 phases of feeding after attachment. In the first phase the major diet is tissue fluid exudate and neutrophil (exudate-neutrophil feeders) which lasts for about 10 days after attachment. Probably between the 10th and 14th day the flea passes through the 2nd phase in which the gut contents show mostly fibroblasts and collagenous materials (connective tissue feeders). The 3rd phase (14 days after attachment) is marked by feeding on whole blood. As in the case of rabbit flea *S. cuniculi* there appears to be an interesting correlation and synchronization of the reproductive activities of the flea and the feeding phases. Tissue feeding (phases 1 and 2) is associated with neosomy (change involving the hypertrophy of the abdomen especially the 2nd and 3rd abdominal segments) while the haematophagous phase initiates reproductive activities of the flea such as mating, ovarian development and egg laying. It is during the 2nd phase (connective tissue feeding phase) that the midgut epithelium

hypertrophies with greater secretory activities, the condition resembling that seen in rabbit flea *S. cuniculi* (Mead-Briggs 1964). This increased secretory activity is probably for efficient utilization of the blood meal for ovarian maturation and egg production. According to Lavoipierre *et al* (1979a) there appears to be a remarkable integration between host inflammatory and repair responses and feeding behaviour, growth and reproduction of the flea and this interaction between the parasite and the host certainly does not appear to be fortuitous. It is replicative and predictable and is never anarchic. These authors also found the dorsal surface of the pinna of the ear to be the specific site for attachment of *T. monositus* and attachment elsewhere results in premature death of the flea. A comparative study showed that feeding behaviour of *T. monositus* is similar whether the host offered *M. musculus* albino or *Peromyscus maniculatus* (the natural host of this flea). However, the intake of neutrophils on the latter host was more during the cellular exudate feeding phase and haematophagy commenced earlier when on *Peromyscus* compared to *Mus*. There is an increase in the rate of neosomy also on *Peromyscus* (Lavoipierre *et al* 1979b).

3. Conclusions

One of the most prominent features that may be noticed in flea-host association is that fleas mostly parasitize hosts which dwell in burrows/nests. This being the case the climatological and/or nutritional requirements of the free living larval stages become important factors in host preference of adult fleas. This prompted many workers to suggest that fleas are nest specific rather than host specific. Rothschild (1966) recognized mainly two life styles of fleas. The closer association with the nest microhabitat (primitive, if it is accepted that the progenitors of fleas were scavengers in the nest of vertebrates) and closer association with the microhabitat of the epidermis of the host, sometimes extending to very prolonged and intimate associations as seen in *Echidnophaga* and *Tunga.*

But with regard to host association of adult fleas for which adaptation to epidermal habitat is also important, three categories may be recognized. The first is exemplified by a type like the rat fleas *X. cheopis*/*X. astia* (Cheopis-type). These are not nest fleas as they spend more time of their adult lives on the host compared to certain species of *Coptopsylla* or *Ceratophyllus*. The former is found on the body of the host for hardly 3 h in its life time and during this period it ingests about 50–100% of its own body weight of blood (Balashov 1984). In contrast to this *X. cheopis*/*X. astia* remain on the body of the host for longer periods, feed very frequently, ingesting each time only very small quantity of blood (usually less than their own body weight). This limited capacity is probably genetical and there is considerable restraint in the extensibility of the abdomen which restricts the quantum of blood it is capable of ingesting. This coupled with the comparatively large sized eggs into which fairly large quantities of yolk material are to be packed and the low protein storage capacity of haemolymph (K G Narayana Pillai and R S Prasad, unpublished results) makes it obligatory on the part of these insects to remain in close contact with the host which would facilitate frequent feeding (Prasad 1986).

The second category is the hormone bound fleas (Cuniculi-type) examples of which are the rabbit fleas and probably the bat fleas. There is no information on the mechanisms involved in the case of bat fleas. In rabbit fleas parasitism has advanced

so much that the fleas are able to breed only if fed on a pregnant host. As it is with all narrow adaptations, it would have its own advantages and disadvantages. It would be interesting to know if these fleas would respond to hormonal changes associated with breeding of vertebrates other than their natural hosts. This association grades into a third and more intimate relationship which may be qualified as Monositus-type, seen in fleas belonging to the genera *Echidnophaga* and *Tunga*. There is no known hormonal involvement as in the previous instance and Lavoipierre *et al* (1979a) state that survival of *T. monositus* is dependent on the host inflammatory and repair responses with which there appear to be remarkable integration of feeding behaviour, growth and reproduction. As was already explained neosomy is dependent on tissue fluid feeding and reproduction on haematophagy. What are the precise physiological mechanisms guiding these two phases? The situation finds some parallel with the condition in ticks. In these cases also species variations in the host do influence blood feeding, rate of blood digestion etc. Are the reproductive functions of the first and the third groups influenced by common factors? Are these dependencies simply for dietary proteins? or are there any specificities for blood proteins or their constituent amino acids?. Milk fed *X. cheopis* and *X. astia* did not develop oocytes indicating that they are not able to utilize casein for ovarian maturation. Among blood proteins, bovine albumin in place of bovine haemoglobin did not trigger maturation (Kamala Bai and Prasad 1976, 1979). Does it mean that these fleas have evolved certain specificities in amino acid requirements? So far there are no experimental results which unequivocally declare that blood from certain species of hosts are nutritionally inferior or certain others superior.

There are indications of preferences to certain sites on the epidermal habitat. For example rat fleas *X. cheopis* seem to prefer the posterior half of the body of white rat especially the base of the tail, while *X. astia* prefer anterior half mainly the neck region. The areas preferred by *X. astia* are warmer than those preferred by *X. cheopis*. If there is any other factor influencing this preference is not clear (Prasad 1972). Rabbit fleas *S. cuniculi* choose the rabbit's muzzle and cheeks during the early stages after emergence and later move on to the ears where they become well anchored. *Nosopsyllus fasciatus* preferred the hind quarters of the host while *Leptopsylla segnis* the fore quarters. *T. monositus* show very strict preference for the dorsal part of the pinna of the ear.

From the present analysis it would become clear that nutritional status of the blood is only one of the many factors deciding suitability of a particular vertebrate as a host. Perfect adaptation to a host thus would depend on a proper coordination of factors influencing larval and adult environments. There are still wide lacunae in our understanding of host association of this group of insects.

Are haematophagy and vertebrate host association (specificities) the effect of primary adaptation to the nesting conditions of the host (cause) or vice versa? If the assumption that progenitors of Siphonaptera [probably a boreus-like Mecopteran (Hinton 1958; Rothschild 1975; Schlein 1980)] were primarily associated with the lair or nest of vertebrates (James and Harwood 1969) it might then follow that development of haematophagy and the associated factors like adaptation to host-borne chemical emanations and epidermal habitat were subsequent developments. As mentioned by Waage (1979) the invasion of a nest microhabitat by predator ancestor and the evolution of increasingly intimate host association is a probable scenario for the evolution of Siphonaptera. A nest/burrow habitat would offer 3

major food sources: (i) the detritous (for scavengers); (ii) the arthropod body fluid (prey-predator relationship) and (iii) the vertebrate blood (host-parasite relationship). Fleas have exploited the first and third sources of food offered by the nest/burrow habitat thereby optimizing on the available nutrient source and increasing fitness in the habitat. If this exploitation was directed by the cues correlated with the energy content of the food (theories of optimal diet/optimal foraging) is not known. Except for social insects, the fitness of an insect will be a function of individual survivorship and fecundity (Mitchell 1981). As it is evident from the foregoing account, survivorship of fleas is not influenced by nutrition alone. At least 3 factors in a nest/burrow habitat control survivorship: moisture content affecting mostly the larval stages; nutrient content of the nest/burrow detritus affecting the larval stages and behavioural resistance of host affecting the adult fleas. Fecundity on the other hand is controlled by nutrition. Proteinaceous meal is a must for ovarian maturation and egg production. About 16 (chicken)–23% (human) of protein content, vertebrate blood is nutritionally superior to either arthropod haemolymph [insect haemolymph has about 1–10% of protein (Wyatt 1975)] or plant sap which has practically no proteins. Vertebrate blood diet would thus offer definite advantage in reproductive functions to those which switched over to this diet and a selection at this level would be to the advantage of the organism. Such switching over could be possible under situations of indiscriminate feeding by the insect concerned. According to Emlen (1968) predators will tend to specialize in their diet (not necessarily on usually superior food) when food is abundant and to feed more and more indiscriminately as food becomes scarce. The feed back between the food's use and its value leads towards increased specialization. Change over from predation on arthropods to parasitism on vertebrates has other advantages also. For example, a vertebrate being larger, a single animal can support a large population of the parasite.

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Effect of okra fruit blocks, seeds and pericarp on post-embryonic development of *Earias vittella* (Fab.) in relation to some phytochemicals of selected okra genotypes

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Abstract. Post-embryonic development of the spotted bollworm, *Earias vittella* (Fab.) studied on pericarp, seeds and fruit blocks of 8 okra genotypes, revealed significant differences in larval survival, larval period, pupation and adult emergence among genotypes as well as fruit components. First instar larval survival on fruit components was in the order of fruit blocks > seeds > pericarp > axil. Mean total developmental period was 24·20, 21·33 and 21·09 days on pericarp, seeds and fruit blocks, respectively. Likewise pupation was 25·30, 65·42 and 71·58% on pericarp, seeds and fruit blocks, respectively. Primary phytochemicals, like protein, free amino acids, total sugars, non-reducing sugars and moisture content in pericarps were in higher concentrations in comparison to seeds and whole fruits. These compounds also differed significantly among genotypes. There were positive correlations between the pest survival and these nutritional compounds. Tannins were 0·35, 0·23 and 0·29% in pericarp, seeds and whole fruits, respectively. Tannin content was significantly higher in pericarps of tolerant genotypes and showed significant negative correlation ($r = -0{\cdot}81$) with fruit borer survival.

Keywords. Okra; *Earias*; phytochemicals; pericarp; post-embryonic; genotype; antibiosis; nutrition.

1. Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), an important vegetable grown in India, is attacked by over 37 insect species (Nayar *et al* 1976). Among these, shoot and fruit borer (*Earias vittella* (Fab.)) also popularly known as spotted bollworm of cotton is the most ubiquitous, causing damage to okra fruits to the extent of 90% (Krishnaiah *et al* 1976). Okra fruit components like epicarp, seeds, cut pieces and whole fruit have been reported to affect developmental behaviour and reproductive potential of this pest (Vishwapremi and Krishna 1974a, b). The poor reproductive potential of this fruit borer on epicarp was attributed to less number of free amino acids and lower concentration of water soluble proteins in epicarp when compared to seeds (Mani *et al* 1986). The present study report the influence of different okra fruit components on survival and development of *E. vittella* in relation to some phytochemicals of susceptible and tolerant okra genotypes.

2. Materials and methods

The okra genotypes, viz KS-305 and Line 14–78 which had tolerance and the genotypes, viz Pusa Sawani, Lam hybrid, AC-302, AC-333, Vashalivadu and Sel-2 which

had the susceptible reactions to the attack of this borer under field conditions (Singh 1985) were selected to carryout the following studies.

2.1 *Insect survival and development*

Survival of first instar larvae was studied on 4 different components viz pericarp, seeds, axil (placenta) and fruit blocks (2 cm long) of 4–5 day old fruits in separate aerated plastic containers (10 × 5 cm) in 3 replications. Freshly hatched 10 larvae per container were released and kept in BOD incubator at 30 ± 1°C. The food was changed on alternate days and observations on surviving larvae were recorded after 4 days of their release. In another set, similarly rearing of first instar larvae was carried out till pupation. Data were then recorded on larval, pupal, total (larval + pupal) developmental periods, pupation and adult emergence. Growth indices for larval and total developmental periods were calculated by the following formulae (Sharma *et al* 1982).

$$\text{Larval growth index} = \frac{\text{Pupation (\%)}}{\text{Mean larval period (days)}}$$

$$\text{Total developmental growth index} = \frac{\text{Adult emergence (\%)}}{\text{Mean total developmental period (days)}}$$

2.2 *Phytochemical estimations*

Okra fruits, 4–5 days old, were sampled during last week of August at peak period of insect incidence. Along with whole fruits, pericarp and seeds were oven dried separately at 60°C for 48 h. These were ground in a Willey grinding mill to pass them through 20 mesh seive. Axil portion of the fruit was not collected for phytochemical estimations, because it constitutes less than 10% of the total fruit weight (Singh 1985) and is also of less importance in influencing insect incidence. Moisture content was estimated by drying the fresh fruits and their components in an oven at 70°C for 48 h. Total sugars were extracted in distilled water by following the method of Srinivasan and Bhatia (1953) and estimated according to the method of Yemm and Willis (1954). Reducing sugars were estimated by the method of Somogyi (1952). Free amino acids were extracted according to Barnett and Naylor (1969) and estimated by Yemm and Cocking (1955). For proteins estimation, initially total nitrogen was determined by micro-Kjeldahl's method (AOAC 1970) and nitrogen percentage was multiplied by 6·25. Tannin content was estimated according to the method of Burns (1971). These phytochemicals were expressed as per cent of dry weight of the sample taken. The data were analysed and simple correlations of phytochemicals were worked out with pest survival.

3. Results

3.1 *Insect survival and development*

Table 1 shows that mean survival of first instar larvae was maximum (67·49%) on okra fruit blocks followed by seeds (59·58%), pericarp (28·33%) and axil (10·42%).

Table 1. Survival of first instar larvae of bollworm on different fruit parts of okra genotypes.

Genotype	Larval survival (%)			
	Pericarp	Axil	Seeds	Fruit block
Pusa sawani	26·67 (30·94)	13·33 (21·14)	63·33	66·66
Lam hybrid	63·33 (52·78)	26·67 (30·94)	46·66	76·66
AC-302	36·67 (37·22)	0·00 (3·63)	76·66	86·66
AC-333	33·33 (35·22)	23·33 (28·78)	66·66	76·66
Sel-2	33·33 (35·22)	6·67 (13·49)	66·66	73·33
Vashalivadu	26·67 (30·99)	13·39 (21·14)	70·00	70·00
KS-305	6·67 (12·29)	0·00 (3·63)	43·33	46·66
Line 14–78	0·00 (3·63)	0·00 (3·63)	43·33	43·33
Average	28·33	10·42	59·58	67·49
SEM	(2·23)	(2·46)	3·12	3·24
CD at 5%	(6·78)	(7·47)	9·46	9·84

Figures in parantheses are angular values.

Hence axil portion was least suitable in all genotypes with no larval survival on KS-305, Line 14–78 and AC-302. Larval survival on pericarpic region of tolerant genotypes was specifically poor where it ranged from 0·0–6·67% only. Pericarpic region of Lam hybrid was most suitable, where larval survival was upto 63·33%. Seed portion of all genotypes supported 43·33–76·66% larvae to survive. However, best survival of the larvae was recorded on fruit blocks.

The results of elaborate studies on the post-embryonic development of the pest are given in table 2. Feeding of larvae on pericarp of tolerant genotype KS-305 prolonged total developmental period to the extent of 5 days in comparison to Vashalivadu. None of the larvae survived on pericarp of Line 14–78. The per cent pupation (40) and adult emergence (95) were recorded to be the highest on Lam hybrid. As a result, this genotype also manifested highest total growth index (4·15). Poorest growth index (1·87) was observed on tolerant KS-305. Total developmental period on seeds of different genotypes ranged from 19·15–22·86 days. Tolerant genotypes showed slightly longer developmental period than susceptible ones. Pupation ranged from 20–90·65%, adult emergence 52·50–96·0% and total growth index 1·78–4·93 in different genotypes. Total developmental period on fruit blocks of test genotypes varied between 18·25 (Lam hybrid) and 25·25 (Line 14–78) days. Pupation (30·35%), adult emergence (30·00%) and total growth index (1·00) were also lowest on tolerant genotype Line 14–78. Highest adult emergence (100%) and total growth index (5·48) were recorded on Lam hybrid.

3.2 *Phytochemical variations*

Data on biochemical analysis are given in table 3. The concentrations of phytochemicals in pericarps varied significantly except moisture level, where proteins ranged from 13·85–17·90, free amino acids 2·52–4·05, total sugars 5·00–10·75, reducing sugars 1·75–3·75, non-reducing sugars 3·25–7·08, moisture content 90·85–92·60 and tannins 0·20–0·61% in tested genotypes. In seeds, proteins varied from 13·25–18·37, free amino acids 1·92–3·04, total sugars 4·35–10·50, reducing sugars

Table 2. Effect of feeding on okra fruit components on post-embryonic development of spotted bollworm in okra genotypes.

Fruit component	Genotype	Developmental period (days)			Pupation (%)	Larval growth index	Adult emergence (%)	Total growth index
		Larval $\bar{X} \pm SE$	Pupal $\bar{X} \pm SE$	Total $\bar{X} \pm SE$				
	Pusa sawani	12·42 ± 0·4	11·23 ± 1·6	22·79 ± 2·1	10·70	0·86	90·50	3·80
	Lam hybrid	11·33 ± 0·7	10·67 ± 0·8	22·90 ± 1·4	40·00	3·53	95·00	4·15
Pericarp	Vashalivadu	11·24 ± 0·3	10·63 ± 0·4	22·00 ± 1·6	30·00	2·67	70·80	3·22
	AC-302	11·44 ± 0·6	12·50 ± 1·2	24·74 ± 1·4	35·50	3·10	90·50	3·66
	AC-333	12·86 ± 0·6	11·33 ± 0·7	24·38 ± 1·4	20·50	1·54	60·60	2·49
	Sel-2	13·79 ± 0·2	12·67 ± 1·1	25·79 ± 2·4	15·40	1·12	80·00	3·10
	KS-305	13·63 ± 0·2	12·50 ± 0·4	26·80 ± 0·9	25·00	1·83	50·00	1·87
	Line 14–78	0·00	0·00	0·00	0·00	0·00	0·00	0·00
Average		12·39	11·65	24·20	25·30	2·09	76·77	3·18
	Pusa sawani	9·25 ± 0·2	10·14 ± 0·6	19·75 ± 1·2	65·50	7·00	95·30	4·82
	Lam hybrid	9·45 ± 0·3	12·35 ± 0·8	22·70 ± 1·4	70·40	7·45	90·50	3·98
Seeds	Vashalivadu	8·50 ± 0·2	10·32 ± 0·4	19·15 ± 1·3	85·50	10·06	80·00	4·18
	AC-302	8·20 ± 0·4	10·45 ± 0·3	19·45 ± 1·4	90·00	10·98	96·00	4·93
	AC-333	10·90 ± 0·2	11·50 ± 0·2	22·79 ± 1·6	75·35	6·91	70·40	3·09
	Sel-2	10·33 ± 0·8	10·41 ± 0·4	21·25 ± 2·1	90·65	8·78	94·00	4·42
	KS-305	10·55 ± 0·7	12·65 ± 0·6	22·86 ± 1·3	25·50	2·42	40·60	1·78
	Line 14–78	9·90 ± 0·2	12·70 ± 0·8	22·70 ± 1·8	20·50	2·07	52·50	2·31
Average		9·63	11·31	21·33	65·42	6·96	77·41	3·69
	Pusa sawani	9·75 ± 0·6	12·24 ± 0·7	21·75 ± 2·7	80·25	7·13	80·00	3·33
	Lam hybrid	8·50 ± 0·5	9·50 ± 0·2	18·25 ± 1·3	84·65	9·96	100·00	5·48
Fruit	Vashalivadu	8·50 ± 0·2	9·55 ± 0·2	18·55 ± 1·3	65·40	7·69	96·40	5·20
blocks	AC-302	9·25 ± 0·2	12·20 ± 0·9	22·20 ± 2·6	90·45	9·78	94·00	4·23
	AC-333	8·20 ± 0·2	9·60 ± 0·8	18·63 ± 1·4	85·50	10·43	100·00	5·37
	Sel-2	9·10 ± 0·8	10·15 ± 0·7	20·10 ± 1·7	70·50	7·75	98·50	4·90
	KS-305	11·25 ± 0·1	12·25 ± 0·8	24·00 ± 2·4	65·60	6·73	85·50	3·93
	Line 14–78	12·50 ± 0·3	13·15 ± 0·6	25·25 ± 3·2	30·35	2·43	30·00	1·00
Average		9·63	11·08	21·09	71·58	7·74	85·55	4·18

1·28–5·12, moisture 87·63–91·43 and tannins 0·12–0·39% with significant differences among genotypes. However, in seeds, moisture level did not differ significantly. With regard to biochemical analysis of whole fruits, proteins ranged from 13·35–17·25, free amino acids 2·00–3·40, total sugars 4·46–10·31, reducing sugars 1·29–4·92, moisture 88·00–91·80 and tannin content 0·17–0·49% in different genotypes.

4. Discussion

Pericarpic region of okra fruit is very important from the angle of fruit borer attack, because it directly comes into contact with insect eggs, larvae and even adults. Female moths prefer to lay more eggs on hairy surface of pericarp (Singh 1985). Larvae also initially feed and penetrate through this portion. Irrespective of the genotypes, feeding of larvae exclusively on pericarp resulted in poor survival, longer larval period, less pupation and poor larval growth index in comparison to seeds and fruit blocks. These observations are in accordance with that of Vishwapremi and Krishna (1974a, b) who also reported longer larval period and poor reproductive

Table 3. Quantitative variations in biochemicals of okra fruit components in different genotypes.

Fruit component	Genotype	Protein (%)	Free amino acids (%)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Moisture (%)	Tannin (%)
	Pusa sawani	16·12	2·71	8·50	2·50	6·00	92·60	0·24
	Lam hybrid	17·90	3·12	8·20	2·65	5·55	91·19	0·24
Pericarp	Vashalivadu	16·25	4·04	5·00	1·75	3·25	91·58	0·26
	AC-302	16·00	2·64	9·50	2·42	7·08	91·01	0·20
	AC-333	16·50	2·74	8·50	2·55	5·95	90·90	0·20
	Sel-2	14·65	2·84	10·75	3·75	7·00	91·75	0·42
	KS-305	15·25	2·61	7·50	2·48	5·02	90·85	0·60
	Line 14-78	13·85	2·52	6·50	2·45	4·05	91·79	0·61
Average		15·81	2·90	8·06	2·57	5·49	91·46	0·35
SEM ±		0·14	0·05	0·12	0·06	0·14	N.S.	0·01
C D at 5%		0·40	0·16	0·35	0·18	0·56	—	0·04
	Pusa sawani	15·40	1·92	6·50	2·85	3·65	91·43	0·14
	Lam hybrid	14·65	2·46	7·00	2·55	4·45	89·85	0·20
Seeds	Vashalivadu	18·37	3·04	4·35	1·28	3·07	90·88	0·12
	AC-302	16·25	2·06	6·35	2·75	3·60	87·63	0·19
	AC-333	17·25	2·28	6·25	2·84	3·41	89·23	0·13
	Sel-2	13·25	2·12	10·50	5·12	5·38	90·32	0·31
	KS-305	13·75	2·35	6·25	2·75	3·50	89·00	0·39
	Line 14-78	14·50	2·01	5·25	2·25	3·00	89·15	0·35
Average		15·43	2·27	6·56	2·80	3·76	89·69	0·23
SEM ±		0·13	0·06	0·10	0·08	0·11	N.S.	0·01
C D at 5%		0·39	0·21	0·30	0·21	0·36	—	0·03
	Pusa sawani	15·54	2·00	7·01	2·67	4·34	90·70	0·17
	Lam hybrid	15·64	2·65	7·28	2·80	4·48	88·00	0·21
Whole	Vashalivadu	17·25	3·40	4·46	1·29	3·17	91·30	0·17
fruits	AC-302	16·53	2·10	7·08	2·60	4·48	88·10	0·20
	AC-333	16·78	2·44	6·79	2·74	4·05	90·40	0·17
	Sel-2	13·79	2·46	10·31	4·92	5·39	91·80	0·40
	KS-305	14·56	2·43	6·60	2·66	3·94	88·24	0·48
	Line 14-78	13·35	2·32	5·53	2·38	3·15	88·90	0·49
Average		15·43	2·47	6·88	2·76	4·12	89·68	0·29
SEM ±		0·15	0·04	0·11	0·03	0·12	N.S.	0·01
CD at 5%		0·43	0·12	0·34	0·08	0·35	—	0·04

Table 4. Simple correlation between phytochemicals of okra fruit components and spotted bollworm survival.

	Correlation coefficient (r)		
Phytochemical	Pericarp	Seeds	Fruit blocks
Moisture	0·60	0·58	0·74*
Total sugars	0·45	0·54	0·35
Reducing sugars	0·35	0·39	0·25
Non-reducing sugars	0·55	0·41	0·46
Protein	0·42	0·38	0·36
Free amino acids	0·15	0·11	0·01
Tannin	−0·81*	−0·46	−0·56

*Significant at 5% level.

potential due to larval feeding on pericarp. To support the normal growth of insect, primary plant chemicals (Hsiao 1974) like proteins, amino acids, sugars and moisture contents appeared to be in sufficient amounts in pericarpic region of okra fruits. In addition, concentrations of total sugars, non-reducing sugars and amino acids were higher in pericarp than seeds and whole fruits which indicated better phagostimulatory activity for the pest. Such facts are in accordance with the views of Thorsteinson (1958). But qualitatively pericarp has been reported to be inferior to okra seeds with respect to free amino acids and water soluble proteins (Mani *et al* 1986). However, there is need to investigate quantitative differences among different essential and non-essential amino acids present in pericarp and seeds. Similar to present studies from sugars point of view, Mani *et al* (1986) also did not find pericarp inferior to seeds. Under utilization of nutritional compounds may also be due to the presence of some antinutritional factors in higher amounts in pericarpic portion. Tannin was found to be higher in pericarp (0·35%) than seeds (0·23%) and whole fruit (0·29%). Antibiosis property of tannins have been reported against a number of pests (Bennett 1965; Maxwell *et al* 1967; Feeny 1968). Tannins react with digestive enzymes and other proteins in insects thereby reducing the nutritive value of the ingested foods (Chan *et al* 1978). They may also act as feeding inhibitors (Sharma and Agarwal 1981). This antibiotic compound was further significantly higher in pericarps of tolerant genotypes in comparison to susceptible ones. However, in present studies, tannin content in okra fruits did not exceed 0·49% which may not be sufficient to induce complete antibiosis. Chan *et al* (1978) reported 3·4% tannin in cotton squares of *Heliothis armigera* F. resistant genotypes. Against *E. vittella*, Sharma *et al* (1982) estimated 1·96% tannin in bolls of least suitable genotype. Hence tannin effects may be supplemented through the inferior quality of free amino acids and water soluble proteins in epicarp as reported by Mani *et al* (1986).

With regard to developmental parameters on seeds and fruit blocks, the overall differences between larval period, pupal and total developmental period and growth indices were negligible. However, pupation on fruit blocks (71·58%) was better than on seeds (65·42%). It was probably due to better pupation site or thigmotactic stimulus offered by fruit blocks. Vishwapremi and Krishna (1974a) also reported shortest pupal period and heaviest pupal weight for the larvae reared on fruit cut pieces in comparison to whole fruit, seeds and epicarp. Contrary to this, okra seeds showed better reproductive potential (Vishwapremi and Krishna 1974b; Ambegaonkar and Bilapate 1982).

Moisture content, total sugars, reducing sugars, non-reducing sugars, proteins and free amino acids of pericarp, seeds and whole fruits of okra genotypes were positively correlated with pest survival (table 4). Among these, only moisture content of whole fruit had positive and significant ($r = 0{\cdot}74$) correlation with fruit borer survival. Tannins of pericarp, seeds and whole fruits showed negative correlation with pest survival.

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In vitro studies on the involvement of brain and gonad in the functional differentiation of prostate gland in *Cryptozona belangeri* (Deshayes) (Mollusca: Gastropoda)

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Abstract. Prostate glands from mature and juvenile *Cryptozona belangeri* were cultured *in vitro* for 8 days in single or double association with cerebral ganglia, visceral ganglionic complex and gonads obtained from adult snails. The histology of the glands was studied and compared with those of normal glands and with glands cultured *in vitro* without any organ association. Functional differentiation was not evident in glands cultured in isolation or in single association with gonad or brain. In double associations, involving cerebral ganglia, visceral ganglionic complex and gonad response from the gland was obtained only when the gonad was involved. The cerebral ganglia and visceral ganglionic complex acting through the gonad elicit differential responses from the prostate gland. It was concluded from the *in vitro* studies that the prostate gland may be dependent on a gonadal hormone for functional differentiation, which in turn is under the control of trophic factors released from the brain.

Keywords. *Cryptozona belangeri*; *in vitro* studies; functional differentiation; prostate; cerebral ganglia; visceral ganglionic complex; gonad.

1. Introduction

Regulation of reproductive activities in pulmonates has remained a perplexing problem. Various explanations have been proposed by different workers to account for the reproductive process in molluscs but as pointed out by Golding (1974), the differences in the techniques employed, the diversity of regulative mechanisms and the disarray relating to species variations serve to stress the enormity of the problem. In the absence of well defined endocrine organs in molluscs, the gonad and the neurosecretory cells located in the brain and the optic tentacles are known to be implicated in the control of reproductive activities.

In the brain, the cerebral ganglia influences differentiation of the male germ cells (Guyard 1971), vitellogenesis (Streiff 1967; Choquet 1971) and reproductive tracts (Boer and Joosse 1975). The involvement of the visceral ganglionic complex in reproductive activities has been poorly investigated.

The status of the gonad as an endocrine gland has remained controversial. Histologically there is no evidence to account for the production of hormones in the gonad (Boer and Joosse 1975). However experimental studies embracing castration, transplantations and organ culture methods have shown that the gonad has some effect on the reproductive tract, albumen gland, prostate and female glands (Abeloos 1943; La Violette 1954; Bailey 1973; Runham *et al* 1973).

The major drawback in investigations involving the brain and the gonad is the heavy mortality rate resulting from surgery or castration in shelled pulmonates (Goddard 1960). Removal of the shell or even a sizeable part of it is lethal to the

animal. Further, the gonad is closely apposed to the hepatopancreas, which is invariably damaged during surgery. To overcome this difficulty a recent trend is the usage of *in vitro* methods.

The present study has been undertaken because of the paucity of data on regulative measures of the accessory glands in terrestrial pulmonates and also taking into consideration the value and advantages of using *in vitro* techniques. The experiment is designed to study the influence of the gonad and brain on the functional differentiation of the prostate gland in the terrestrial pulmonate snail *Cryptozona belangeri* (Deshayes).

2. Material and methods

2.1 *In vitro studies*

The millipore filter organ culture technique devised by Grobstein (1955) and Auerbach (1960) was adopted in cultivating the prostate gland *in vitro*. The procedure of Ball and Auerbach (1961) was followed for the preparation of the millipore assembly. A plexiglass strip ($32 \times 6 \times 2$ mm) possesing a central hole of 3 mm diameter was pasted with a round disc of millipore filter paper to obtain an open well of 3 mm diameter and 1 mm depth. The plexiglass strip was lowered on to the depression of the tissue culture dish of Grobstein (1955) type (figure 1).

2.2 *Culture medium*

With the exception of a few modifications indicated below, the medium was similar to that used by Burch and Caudros (1965) for *Helix aspersa*. The molluscan tissue extract in the culture medium was replaced by chick embryo extract to remove the possible interference of homologous proteins in the molluscan tissue extract (Rajan and Sriramulu 1979). Hedon-Fleig saline (Lockwood 1961) was used for the survival of the explants. Tricine was employed as a buffer (Bailey 1973). Table 1 gives the composition of the culture medium used in the present study.

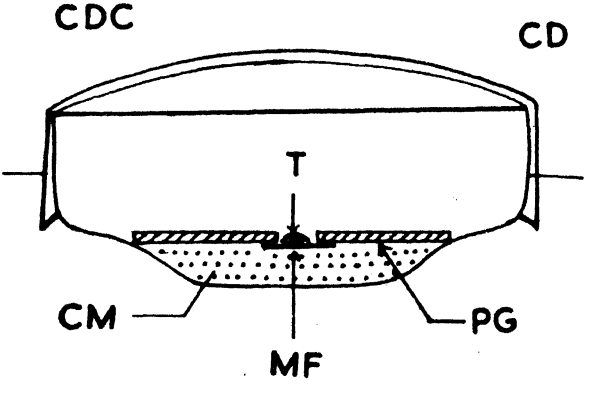


Figure 1. Sectional view of the millipore culture set up. (CD, Culture dish; CDC, culture dish cover; CM, culture medium; MF, millipore filter paper; PG, plexiglas; T, explant tissue).

Table 1. Medium adopted for culture of prostate in *C. belangeri*.

	(ml)
Medium 199 + 0·5% peptone	500
*Brain heart infusion (Difco)	20
S.L. Broth	20
M.9 stock solution A	8
M.9 stock solution B	8
*Salt solution C	100
*Sodium bicarbonate (4 g/l)	100
Calf-foetal serum (Difco)	40
*Chick-embryo extract (Difco)	40
Double distilled water	403
*Tricine (1·25 molar aqueous solution)	25
Antibiotic mixture	8
S.L. Broth	(g)
Casein hydrolysate	10
Yeast extract	5
$K_2H\,PO_4$	6
Diammonium hydrogen citrate	2
Glucose	20
Sodium acetate (hydrated)	25
Glacial acetic acid	1·32 ml
Salt solution	5 ml
Tween 80	1 g
Made up to 100 ml with double distilled water	
Stock solution A	(g)
$Na_2H\,PO_4$ (anhydrous)	75·0
KH_2PO_4 (anhydrous)	37·5
Made up to 1000 ml with double distilled water	
Stock solution B	
NaCl	50·0
$MgSO_4$	20·0
NH_4Cl	100·0
Made up to 1000 ml with double distilled water	
Salt solution C	
NaCl	36·0
KCl	1·0
$MgSO_4$	2·0
Made up to 1000 ml with double distilled water	
Salt solution for SL broth	
$MgSO_4 \cdot 7\,H_2O$	11·5
$MnSO_4 \cdot H_2O$	2·86
Made up to 1000 ml with double distilled water	
Fungizone	50 mg
Penicillin	1,000,000 IU
Streptomycin	1·0/1000 ml

*Modifications introduced in the culture medium adopted by Burch and Caudros (1965).

2.3 *Preparation of explants*

Healthy snails of weight groups of 2–3 g (juvenile) and 6–8 g (mature) were washed in running water to remove dirt and soil. The shell was removed with sterilized scissors and the body wiped with moistened sterilized cotton and 70% alcohol. The unanaesthetized snails were then dissected in Hedon-Fleig saline for the removal of the prostate gland. It was severed into small bits measuring $2 \times 2 \times 1$ mm each. Each piece was transferred to an unit of the millipore filter assembly. The nutritive culture medium was introduced into the depression in the culture dish.

2.4 *The associated organs in the culture*

The associated organs were obtained from mature snails. The brain was detached from the surrounding tissues in Hedon-Fleig saline. The brain was divided into two halves, one comprising the dorsal cerebral ganglia and the other the ventral visceral ganglionic complex (pleural, parietal, pedal and visceral). The gonad was also removed and separated from the associated hepatopancreas. The components of brain and gonad were then used either singly or in combination for association with the prostate gland *in vitro* (tables 2 and 3).

2.5 *Maintenance of culture*

The cultures were maintained for 8 days at 25°C in a BOD incubator in saturated humid condition with a gas phase of 95% O_2 and 5% CO_2. The medium and gas phase were changed on alternate days. Frequent observations through microscope were made to detect any sign of contamination and to note the viability of the tissue.

Table 2. Prostate explant of *C. belangeri* and the associated organs maintained in culture.

Explant	Organs cultured in association with the explant
Heterologous association involving single associated organ	
Juvenile prostate	Control
Juvenile prostate	Mature gonad
Juvenile prostate	Mature cerebral ganglia
Juvenile prostate	Mature visceral ganglionic complex
Heterologous association involving double associated organs	
Juvenile prostate	Mature gonad and cerebral ganglia
Juvenile prostate	Mature gonad and visceral ganglionic complex
Juvenile prostate	Mature cerebral ganglia and visceral ganglionic complex

Table 3. Prostate explant of *C. belangeri* and the associated organs maintained in culture.

Explant	Organs cultured in association with the explant
Homologous association involving single associated organ	
Mature prostate	Control
Mature prostate	Mature gonad
Mature prostate	Mature cerebral ganglia
Mature prostate	Mature visceral ganglionic complex
Homologous association involving double associated organs	
Mature prostate	Mature gonad and cerebral ganglia
Mature prostate	Mature gonad and visceral ganglionic complex
Mature prostate	Mature cerebral ganglia and visceral ganglionic complex

.6 *Histology*

The tissues maintained in culture were fixed in aqueous Bouin's dehydrated in scending grades of alcoholic series, cleared in xylol and embedded in paraffin wax 58–60°C). Sections were cut at 6 μ thickness and these were deparaffinised and tained in Heidenhain's iron haematoxylin, counterstained with aqueous eosin and nounted in Canada balsam.

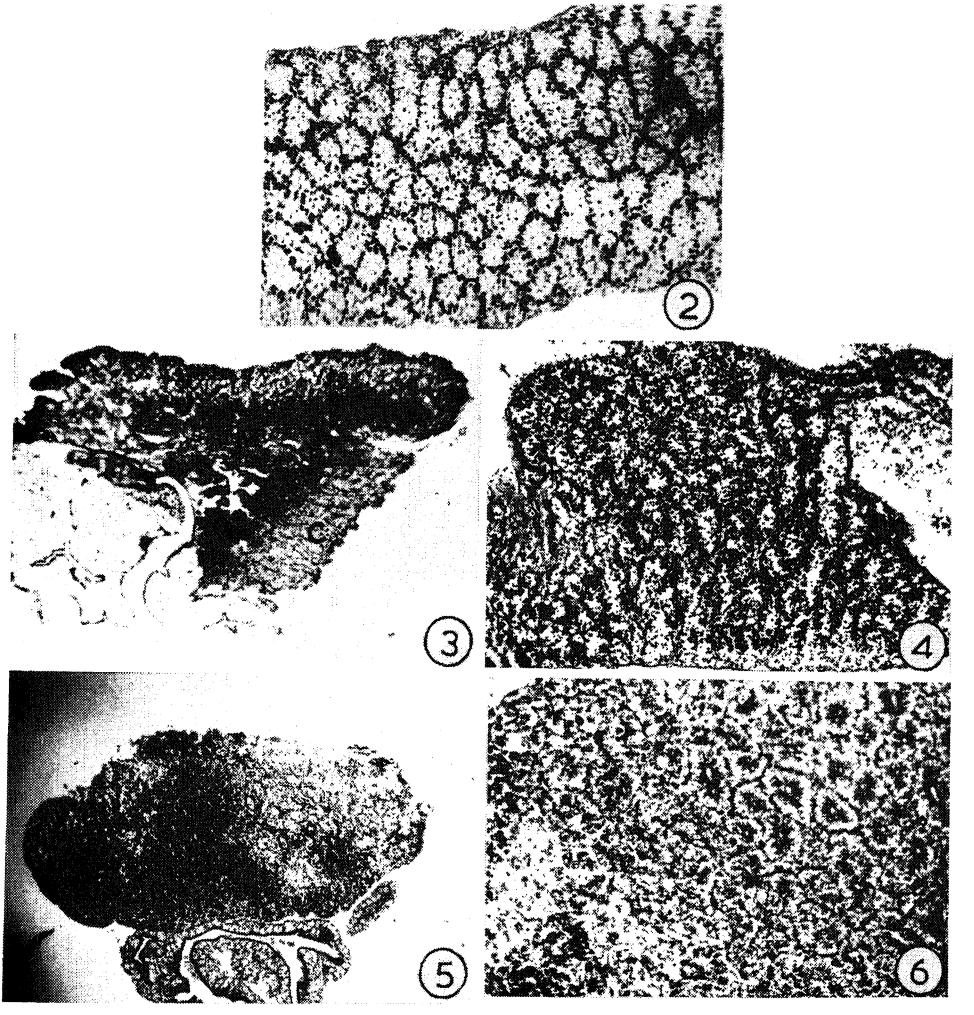
. Observations

.1 *Gross morphology of the prostate gland*

n an immature or reproductively inactive snail, the prostate appears as an indistinct, ale white, thread like structure, running along the dorsal surface of the sperm-viduct. In a mature snail the prostate attains its maximum growth in size, appearing s a separate elongated organ, slightly yellow and closely apposed to the sperm-viduct. This organization of the prostate is retained till the end of the reproduc-ively active period and thereafter the gland involutes and reverts back to its uvenile state.

.2 *Histology of the prostate gland*

.2a *Juvenile*: The small prostate gland cells occur in abundance and lie scattered niformly throughout the tissue in the juvenile snail. The small spherical nuclei ppear distinct. They are centrally placed within the cells and are surrounded by a omogenous thin layer of cytoplasm, with no trace of secretory products (figure 2).



Figures 2–6. **2.** Section through the prostate gland of juvenile snail showing the onset of differentiation of the tubules of the gland (× 80). **3.** Section through the prostate gland of adult snail showing maximum growth of the gland. Note the compact organization of the gland, its shape and its regional differentiation into an outer light area (C) and an inner deeply staining area (M). A thin connective tissue layer surrounds the gland (× 25). **4.** Section through the prostate gland of juvenile snail cultured in isolation. Note the poor organization of tissue (× 80). **5.** Section through the adult prostate gland cultured in isolation. Note necrotic patches towards periphery and deterioration in organization compared to figure 4 (× 25). **6.** Enlarged view of a portion of figure 5 showing disrupted tubules (× 80).

3.2b *Adult*: Based on the histoarchitecture of the adult prostate gland 3 distinct regions can be made out, viz (i) a thin connective tissue layer enclosing the entire gland; (ii) glandular cells of the prostatic tubules and (iii) the lumen enclosed by the glandular cells lined by epithelium.

Based on staining properties, two zones can be made out in the gland: (i) an outer light zone in which tubules are loosely arranged with lightly staining cells, and (ii) a

deeply staining central region in which the tubules are compactly arranged. In these, the cells appear pearshaped, and the broad distal ends of the cells face outwards while the narrow proximal ends face lumen of the tubule. The nuclei are oval, located in the broader end of the cells. The chromatin material stains intensely with haematoxylin and the cell cytoplasm is filled with secretory granules which tend to distend the cell walls (figure 3).

3.3 *Prostate gland cultured in isolation*

3.3a *Juvenile*: Prostate glands cultured in isolation appear much reduced in their size and many of the cells, particularly towards the peripheral region of the gland, appear empty with shrunken cytoplasm (figure 4).

3.3b *Adult*: Disruption in the general organization is evident. Patches of necrotic tissue are visible. Nuclei lie scattered in the disintegrated cytoplasm. Tubular organization is noticed only within the region nearer to the lumen. The tubules are, however, small. The sparse secretory products stain very lightly (figures 5 and 6).

3.4 *Prostate gland in association with gonad*

3.4a *Juvenile*: The gland shows a tendency towards degeneration. The central region is the worst affected, where there is a marked reduction in the number of cells. The nuclei appear pycnotic and the cytoplasm is devoid of secretory products (figure 7).

3.4b *Adult*: Deterioration is evident and the characteristic organization of the gland remains disrupted with no trace of tubules. Cell boundaries appear broken resulting in the cytoplasmic content being dispersed among the tissues. Large gaps and spaces are interspersed between the cells (figures 8 and 9).

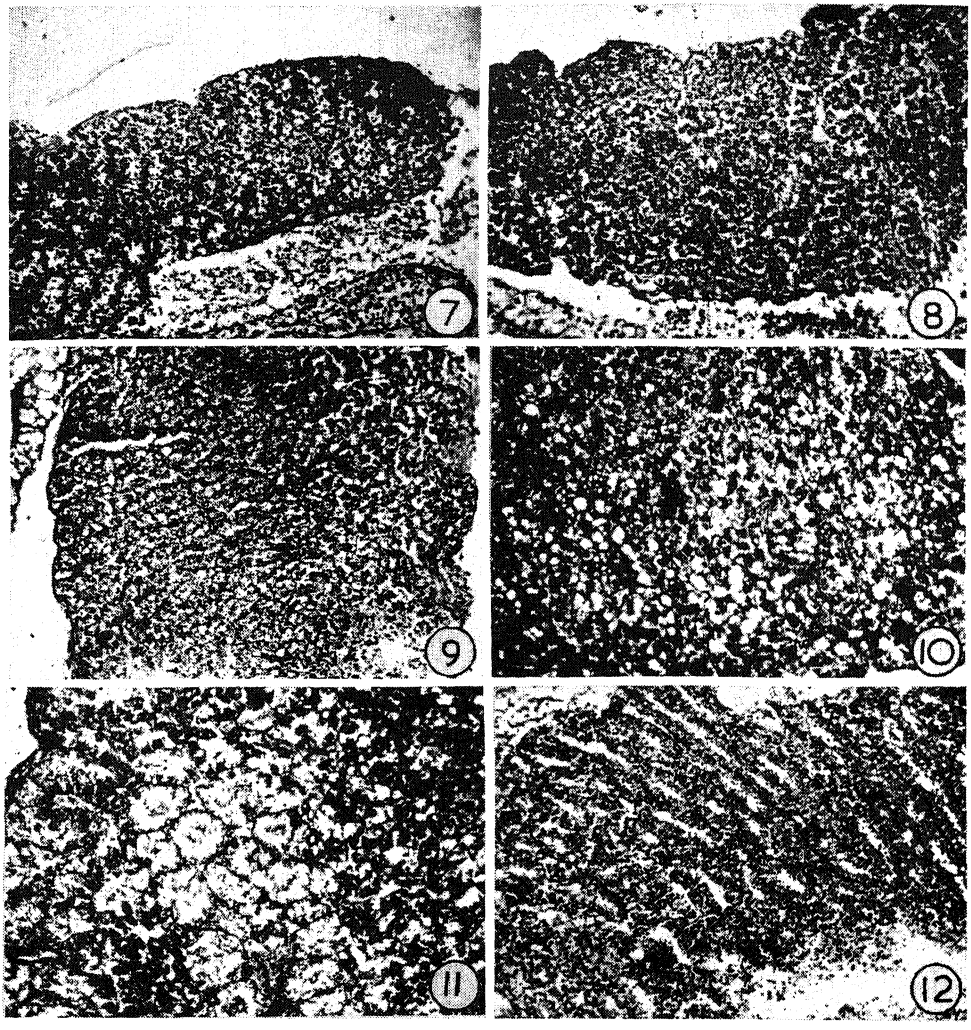
3.5 *Prostate gland in association with cerebral ganglia*

3.5a *Juvenile*: The cytomorphology of the juvenile gland closely approximates that of glands cultured in isolation. Dead tissue areas with unhealthy cells testify to the inability of ganglia to maintain the normal organisation of the juvenile prostate gland.

3.5b *Adult*: Tubular organisation is absent and the central tissue appear vacuolated. A distinct deviation from the normal histology of the gland persists although a slight improvement over the glands cultured in isolation is evident (figure 10).

3.6 *Prostate gland in association with visceral ganglionic complex*

3.6a *Juvenile*: The glandular cells show an increase in their volume. The cell limits are clearly delineated and the cells exhibit a tendency to be grouped together rather than to remain dispersed.



Figures 7–12. **7.** Section through juvenile prostate gland cultured with gonad. Note atrophy of the gland with poor cytological features (×25). **8.** Section through adult prostate gland cultured with gonad. Note absence of tubules and disrupted histology (×25). **9.** Enlarged view of figure 8 showing the disposition of the disorganized tubules (×80). **10.** Section through adult prostate gland cultured with cerebral ganglia. Note vacuolated cells towards the centre (×80). **11.** Section through adult prostate gland cultured with visceral ganglionic complex. Note peripheral tubules with hypertrophied cells. The secretory products of central tubular cells are apparent in the luminae. Cells are not distinct (×80). **12.** Section through juvenile prostate gland cultured with gonad and cerebral ganglia. Note transformation of juvenile histology of gland to adult histology. Tubules have cells with abundant secretions surrounding central lumen (×80).

3.6b *Adult*: A marked change is evident in cells comprising the peripheral tubules which show hypertrophy with secretory products concentrated more towards the narrower ends facing the lumen. Secretory granules are visible in the large luminae (figure 11).

3.7 *Prostate gland in association with gonad and cerebral ganglia*

3.7a *Juvenile*: A complete transformation of the juvenile to adult tissue organisation is encountered when juvenile prostate gland is cultured with gonad and cerebral ganglia. The tubules resemble those found in adult sections taken *in vivo*. The cells are filled with secretory products and are arranged around a central lumen. Peripheral nuclei are distinct (figures 12 and 13).

3.7b *Adult*: In a major part of the gland, a tubular arrangement is visible and the cytoplasm contains secretory products which stain deeply. The histology of the gland closely approximates with that of normal adult glands (figures 14 and 15).

3.8 *Prostate gland in association with gonad and visceral ganglionic complex*

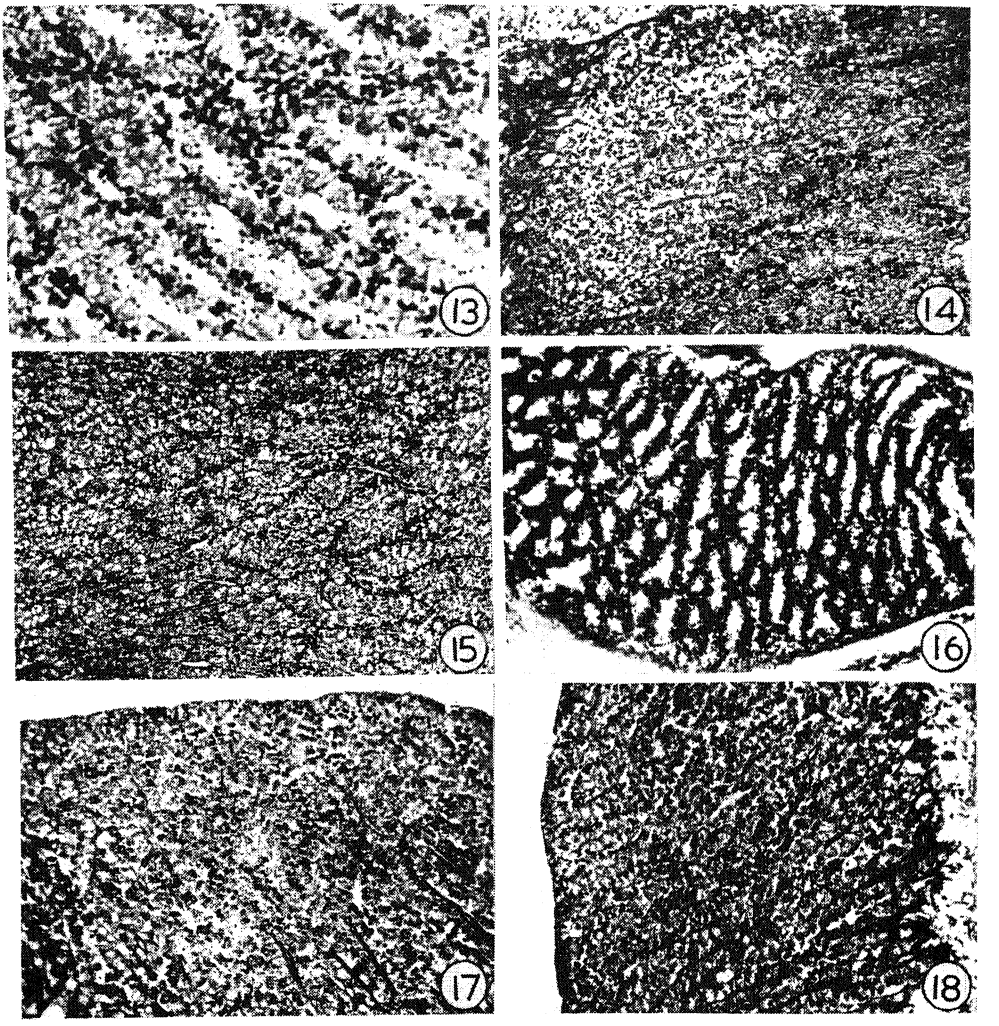
3.8a *Juvenile*: A precocious response of the juvenile gland is met with when associated with adult gonad and visceral ganglionic complex. The gland cells aggregate to form tubules but still retain their spherical shapes. Further, the cytoplasm shows intense secretory activity and the elaborated product considerably increases the size of the cytoplasm. The stress is on the functional activity rather than on structural organization (figure 16).

3.8b *Adult*: Regional differentiation in the histomorphology of the gland is seen. A major portion of the gland away from the duct has hypertrophied glandular cells, which stain deeply and are arranged in ill-defined tubules. Towards the duct, the cell membranes of the cells are disrupted showing released material in the lumen. A few cells have pale and vacuolated cytoplasm with few granules (figures 17 and 18).

4. Discussion

The prostate gland in *C. belangeri* shows close correlation with growth, maturation and reproductive activity of the snail. The functional differentiation of the gland can be deduced from the cytomorphological changes that take place within its glandular cells. The observations recorded in the present study are the result of associations carried out *in vitro* of the juvenile and mature prostate glands with the gonad, cerebral ganglia and visceral ganglionic complex in single or double combinations. This implies a direct action of the associated organs on the gland and also as the active substance emitted by the associated organs acts by diffusing into the culture milieu and there is no actual physical contact between the associated organ and the prostate gland. The histology of the glands thus associated, when compared to those of normal glands and with those glands cultured in the absence of any organ association, reveals striking facts.

The prostate gland in *C. belangeri*, in the absence of any hormonal support, shows poor degree of organization in cultures. This applies to both juvenile and adult glands. This incapacity to function and differentiate under anhormonal conditions is not restricted to the prostate gland alone but has been reported for the dart sac in *C. belangeri* (Megdalene 1981), the male genital tract in *Calyptrae sinensis* (Streiff 1966), penis in *Crepidula fornicata* (Le Gall 1981; Le Gall and Feral 1982) and



Figures 13–18. 13. Enlarged view of figure 12 showing arrangement of cells within tubules. **14 and 15.** Sections through adult prostate gland cultured with gonad and cerebral ganglia. Note regions of actively secretory cells in contrast to areas of tissue with no activity. **16.** Section through juvenile prostate gland cultured with gonad and visceral ganglionic complex showing similarity in organization to figure **13.** However, note the increase in cytoplasmic volume in the spherical cells (× 80). **17 and 18.** Sections through adult prostate gland, cultured with gonad and visceral ganglionic complex showing regional differentiation in cytomorphology. Note the active hypertrophied cells arranged within tubules (figure 17) and vacuolated cells near the duct (figure 18) (× 80).

Note: Section referred to in figures **2–18:** Bouin's fluid, 6 μ Heidenhain's iron haematoxylin counter stained with aqueous eosin.

common duct in *Agriolimax reticulatus* (Bailey 1973). The present result only reflects the view of Highnam and Hill (1979) that development of the reproductive tract is controlled by hormones.

The prostate gland, cultured with gonad from adult snails, showed rapid deterioration in its organization and its functional activity was disrupted. This would

indicate that the gonad inhibits the structural organization and functional integrity of the gland. These results are in contrast to those of castration, transplantation and organ culture experiments, carried out by various workers. Castration in limacid and arionid slugs resulted in negative effects in the albumen and hermaphrodite duct (Abeloos 1943; La Violette 1954). The effects of castration on the activity of the multifid gland in *Helix aspersa* was studied and the role of gonad in maintaining its activity was established (Gomot 1976). In *A. reticulatus*, undifferentiated tracts when transplanted into male phase slugs, showed differentiation of prostate glands, while oviductal and albumen glands remain undifferentiated. However, transplantation of undifferentiated tracts into the haemocoel of egg-laying animals led to the enlargement of the female parts of the tract, while the prostate remained undeveloped. Castration of young specimens resulted in a cessation of development of both the prostate and oviductal glands. This suggests that the production of the male and female hormones had been impeded (Runham *et al* 1973). Immature penial complexes of *A. reticulatus* implanted into mature slugs, displayed growth and differentiation dependant on the stage of the host (Wijdenes 1981). Implants never developed in castrated animals. Bailey (1973) using *in vitro* techniques, has also suggested the implication of the gonad in the control of the prostate in the same slug. However, in Basommatophora, castration experiments have indicated that the development and activity of the accessory sex organs are not under endocrine control of the gonad (Brisson 1971; Brink *et al* 1979; Boer *et al* 1976). In the face of the overwhelming evidences supporting the presence of a gonadal factor in stylommatophoran pulmonates, the absence of such a gonadal factor cannot merely be deduced by direct association of the prostate with the gonad in culture. The possibility, that the poor histological picture of the gland, in single association with the gonad, could be due to the lack of a trophic factor, cannot be ruled out. In fact, in an animal which is strongly dependent on external conditions of temperature and moisture for its reproductive activity, the mediation of the nervous system in the seasonal activity of its reproductive system is strongly indicated.

The direct association of the prostate gland with the cerebral ganglia appears to have no impact on the histology of the gland in *C. belangeri*. A number of evidences can be cited wherein the cerebral ganglia influence reproductive activity, but in the majority of these, the effects (of the principles) are usually related in some way with those of the gonad or the tentacle. Direct action of the principles of cerebral ganglia has been suggested in prosobranchs (Joosse 1979) and in snails (Geraerts and Bohlken 1976; Goudsmit 1975, 1978). The principles of cerebral ganglia in all these molluscs appears to affect either gametogenesis or the female organs. A direct action of cerebral ganglia on male accessory organs has not been reported till now.

A distinct change in the appearance of the gland is evident when cultured with visceral ganglionic complex. This is particularly noticeable in the adult glands, in which the glandular cells appear vacuolated and devoid of granules. Probably some visceral ganglionic principle is responsible for the unconditional liberation of secretory granules from these cells, giving them an exhausted appearance. In the immature glands, apart from a slight increase in cytoplasmic volume no remarkable change is evident.

Since associations of the prostate gland with the brain or the gonad failed to produce positive results, interactions between these two endocrine components were investigated. Recent literature on pulmonate regulative mechanisms strengthens this

view of trophic control. Runham *et al* (1973) concluded from results on *Agriolimax* that male and female parts of the reproductive tract are under separate hormonal control. Probably the sources of the two hormones are the dorsal bodies and the neurosecretory cells in the cerebral ganglia (Geraerts and Algera 1972), and these hormones influence the reproductive tract via the gonad (Boer and Joosse 1975). A relation between brain-gonad axis and photoperiodically stimulated sexual maturation in the slug *Limax maximus* has been studied (McCrone and Sokolove 1979; McCrone *et al* 1981). In the marine pulmonate *Onchidium verruculatum*, it has been shown that a male phase hormone and a female phase hormone are secreted by the cerebral ganglia during different phases of the protandric reproductive cycle (Nagabhushanam and Deshpande 1981). These hormones have been observed to exert their effects on development and functioning of the male accessory sex organs via the gonad. An androgenic influence of the cerebral ganglia on the gonad has also been found in *Helix aspersa* (Guyard 1971). In a review of pulmonate endocrinology, Boer and Joosse (1975) concluded that two gonadal factors perhaps operate separately in the control of male and female lines. Further, it was thought that the dorsal body hormone possibly controls the female factor of the gonad and a neurohormone from neurosecretory cells in the cerebral ganglia controls the male factor.

The results obtained in *C. belangeri*, by the culture of the prostate gland with cerebral ganglia and gonad, is in conformity with the scheme proposed by Boer and Joosse (1975). The cerebral ganglia of adult snails, in conjoint action with the gonad, completely transforms the juvenile appearance of the gland into an organized adult form. This high degree of organisation is in contrast to that of juvenile glands, with cerebral ganglia and gonad in single association. In the adult gland the degree of organization and functional activity appears to be of a high order. These results suggests that the cerebral ganglia has a trophic effect on the gonad which is probably induced to produce a hormone responsible for the growth and edification of the glandular cells.

When cultured with the gonad and visceral ganglionic complex, the juvenile prostate gland in *C. belangeri* shows a precocious response in its secretory activity, but apparently does not alter the shape of the cells. In the adult glands subjected to a similar association, the secretory material appears to spill over into the lumen, and in some cases, empty vacuolated cells which have released almost all their materials into the lumen, can be seen. Apparently, the visceral ganglionic complex appears capable of inducing the gonad to release its hormone; in addition its effect on the prostate gland also seems to be direct. This effect, as previously stated, would be to cause exocytosis of the secretory material and could account for the differences encountered, in associations involving gonad and cerebral ganglia and that of gonad and visceral ganglionic complex.

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Life and fertility tables of *Achaea janata* Linnaeus on castor

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Abstract. The life fecundity tables were constructed when *Achaea janata* Linnaeus reared on castor leaves in the laboratory. The net reproductive rate (R_0) was 467·57 females/female/generation in a generation time of 37·99 days. The intrinsic rate of natural increase (r_m) was 0·1618 female/female/day. In the stable age-distribution, 99% contribution was made by immature stages.

Keywords. Life and fertility tables; *Achaea janata*.

1. Introduction

The castor semilooper, *Achaea janata* Linnaeus is one of the important pests of castor crop in many states of India. The intrinsic rate of increase is a basic parameter which an ecologist may wish to establish for an insect population. Birch (1948) defined it as actual rate of increase of population under specified constant environmental conditions in which space and food are unlimited when there are no mortality factors other than physiological ones. According to Howe (1953), a life-table can be constructed by following the life history of a group of insects from their birth (egg laying) to emergence of adults and recording all deaths as they occur together with sex of those which die in as adult. Therefore, an attempt was made to study life fecundity tables for *A. janata* when reared on castor leaves.

2. Materials and methods

The method used in the present study was similar to those used for *Heliothis armigera* (Hubner) when reared on pigeonpea (Bilapate *et al* 1983). To construct the life fecundity tables, 200 eggs were placed in 10 plastic boxes in batches of 20 each. After hatching, all the larvae were reared individually on castor leaves. Fresh food was supplied daily. Observations on hatching, larval and pupal development, successful adult emergence, fecundity and age specific mortality in eggs, larvae, pupae and adults were made daily. For determining the specific fecundity, all the adults emerged on a particular day were transferred to a separate cage for egg laying. The healthy castor twig was kept in the cages as oviposition site. Considering the sex ratio 1 : 1 (Southwood 1968), the number of eggs laid per female were divided by two to get the number of female births (m_x).

The following column headings proposed by Birch (1948), elaborated by Howe (1953) and Atwal and Bains (1974) were used for the construction of life-fecundity tables under laboratory conditions: x—pivotal age in days; l_x—survival of females at age x; m_x—age schedule for female births at age x.

The values of x, l_x and m_x were calculated from the data on life tables. The sum of

the products $l_x m_x$ is the net reproductive rate (R_0). R_0 is the rate of multiplication of the population in each generation measured in terms of females produced per generation. The number of times a population would multiply per generation was calculated by the formula,

$$R_0 = \Sigma l_x m_x.$$

3. Results and discussion

The results in table 1 indicate that survival of *A. janata* was 184, 177 and 170 eggs, larvae and pupae in a cohort of 200 eggs. A cohort of 200 eggs resulted in the emergence of 89 females and 81 males.

The life-table and age specific-fecundity for *A. janata* indicated that the survival of immature stages was 0·85 (based on one individual) individual within a pivotal age of 32 days (table 2). The pre-oviposition period was 3 days i.e. from 33–35 days of pivotal age. The number of eggs laid per female were divided by two to get the number of female births (m_x). The female births was highest (129·75 female offspring) on the first day of oviposition of the female (36th day of pivotal age) and decreased slowly thereafter. The first female mortality occurred on 7th day ($l_x = 0{\cdot}80$) after emergence of adult when the culture was in the 41st day of pivotal age. The mortality rate of female slowly declined thereafter. Thus, the females oviposited for 9 days. The net reproductive rate (R_0) of female was 467·57 female/female/generation. The results on the mean length of generation, intrinsic rate of increase in numbers and finite rate of increase in numbers of *A. janata* are presented in table 3. The approximate generation time (T_c) was found to be 38·38 days. The arbitrary value for intrinsic rate of natural increase (r_c) was 0·1602 female/female/day. The precise generation time was 37·99 days while the finite rate of increase in numbers was 1·18 female/female/day. The corrected innate capacity for increase in numbers (r_m) was calculated by using the formula, $\Sigma e^{7-r_m \times l_x m_x} = 1096{\cdot}6$.

The two trial values of 1157·12 and 792·96 were plotted on horizontal axis against their respective arbitrary r_c values, 0·16 and 0·17 on vertical axis and the corrected r_m was calculated as 0·1618 female/female/day by interpolation method. The stable age-

Table 1. The survival of life stages of *A. janata* on castor leaves.

	Surviving No.			Sex	
	Egg stage (0–3 days)	Larval stage (4–20 days)	Pupal stage (21–32 days)	Female	Male
	18	17	16	10	6
	17	17	17	10	7
	19	19	18	8	10
	20	18	17	7	10
	18	18	18	10	8
	17	17	17	10	7
	18	17	17	11	6
	19	17	15	7	8
	19	19	18	8	10
	19	18	17	8	9
Total	184	177	170	89	81

Table 2. Life-tables (for females) are age specific fecundity for *A. janata* on castor leaves.

Pivotal age (days) (x)	Life table for female births (L_x)	(m_x)	Age schedule for female births ($L_x m_x$)	($L_x m_x x$)
0–32	0·85		Immature stages	
33–35	0·85		pre-oviposition period	
36	0·85	129·75	110·28	3970·35
37	0·85	88·70	75·39	3789·61
38	0·85	89·75	76·28	2898·92
39	0·85	78·90	67·06	2615·53
40	0·85	66·40	56·44	2257·60
41	0·80	53·05	42·44	1740·04
42	0·72	40·93	29·46	1237·72
43	0·56	18·25	10·22	349·46
44	0·42	0·0	0·00	0·0

$\Sigma L_x m_x = \Sigma L_x m_x x$

$R_0 = 467{\cdot}57 = 17{,}949{\cdot}23$

Table 3. Mean length of generation, innate capacity and finite rate of increase in numbers of *A. janata* on castor leaves.

Population growth statistics	
Mean length of generation	
$T_c = \dfrac{\Sigma L_x m_x x}{R_0}$ $\dfrac{17949{\cdot}23}{467{\cdot}57}$	38·38 days
Innate capacity for increase in numbers	
$r = \dfrac{\log_e R_0}{T_c}$ $\dfrac{6{\cdot}147491}{38{\cdot}38}$	0·1602 ♀/♀/day
Arbitrary $r_c = 0{\cdot}16$ and $0{\cdot}17$ corrected $r_m \Sigma e^{7-} rm_x L_x m_x = 1096{\cdot}6$	0·1618 ♀/♀/day
Corrected generation time	
$T = \dfrac{\log_e R_0}{r_m}$ $\dfrac{6{\cdot}1475491}{0{\cdot}1618}$	37·99 days
Finite rate of increase in numbers $\gamma = \text{anti}\log_e r_m$	
Weekly multiplication $(1{\cdot}18)^7$	3·18
Doubling time $\log_e \dfrac{0{\cdot}6931471}{0{\cdot}1618}$	4·28/day

distribution of *A. janata* was worked out when reared on castor leaves (table 4). On reaching stable age-distribution, the population in its various stages viz the egg, larva, pupa and adult contributed to the tune of 49·06, 48·06, 2·50 and 0·34%

Table 4. Calculated stable age distribution of *A. janata* on castor ($r_m = 0{\cdot}1618$).

Pivotal age (x)	L_x	$e^{-r_m x}(x+1)$	$Lxe^{-r_m(x+1)}$	Percentage distribution	Percentage contribution
0	1	0·8506	0·8506	15·38	
1	1	0·7235	0·7235	13·08	
2	1	0·6154	0·6154	11·13	49·06 eggs
3	1	0·5235	0·5235	9·47	
4	1·00	0·4453	0·4453	8·05	
5	0·98	0·3787	0·3712	6·72	
6	0·96	0·3222	0·3093	5·59	
7	0·96	0·2741	0·2631	4·76	
8	0·95	0·2331	0·2215	4·00	
9	0·94	0·1983	0·1864	3·37	
10	0·94	0·1687	0·1585	2·87	
11	0·94	0·1435	0·1349	2·44	
12	0·93	0·1220	0·1135	2·05	48·06 larvae
13	0·92	0·1038	0·0955	1·73	
14	0·91	0·0883	0·0803	1·45	
15	0·90	0·0751	0·0675	1·22	
16	0·90	0·0639	0·0575	1·04	
17	0·10	0·0543	0·0490	0·89	
18	0·89	0·0462	0·0411	0·74	
19	0·88	0·0393	0·0346	0·62	
20	0·87	0·0334	0·0291	0·53	
21	0·87	0·0284	0·0244	0·44	
22	0·85	0·0244	0·0205	0·37	
23	0·85	0·0205	0·0175	0·32	
24	0·85	0·0175	0·0149	0·27	
25	0·85	0·0149	0·0127	0·23	
26	0·85	0·0127	0·0108	0·19	
27	0·85	0·0108	0·0091	0·16	2·50 Pupae
28	0·85	0·0092	0·0078	0·14	
29	0·85	0·0078	0·0066	0·12	
30	0·85	0·0066	0·0056	0·10	
31	0·85	0·0056	0·0048	0·09	
32	0·85	0·0048	0·0041	0·07	
33	0·85	0·0041	0·0035	0·0633	
34	0·85	0·0035	0·0029	0·0524	
35	0·85	0·0029	0·0025	0·0452	
36	0·85	0·0025	0·0021	0·0379	
37	0·85	0·0021	0·0018	0·0325	
38	0·85	0·0018	0·0015	0·0271	0·34 Adult
39	0·85	0·0015	0·0014	0·0253	
40	0·82	0·0013	0·0011	0·0198	
41	0·76	0·0011	0·0008	0·0144	
42	0·64	0·0009	0·0006	0·0108	
43	0·49	0·0008	0·0004	0·0072	
44	0·42	0·0007	0·0003	0·0054	
			5·5309		

respectively. The type of life-tables developed by Birch (1948) can be used to obtain growth statistics that can be used for both qualitative evaluation of rearing procedures and to estimate the projected potential increase of insects reared on different food commodities. The innate capacity of increase has not been quantified for many insects (Wellik and Pedigo 1978).

According to Bilapate (1987), the net reproductive rate of *A. janata* on castor leaves was 524·78, 382·52 and 242·70 female/female during I, II and III generation under laboratory conditions. The innate capacity for increase in numbers and mean generation time was 0·1692 and 37·01 days during first generation. The growth parameters namely survival of immature stages, contribution towards the female births and net reproductive rate yielded lower value in the present investigations during the rearing of *A. janata* on castor leaves. This might have attributed to the effect of temperature during the period of investigations.

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Cadmium induced vertebral deformities in an estuarine fish, *Ambassis commersoni* Cuvier

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Abstract. *Ambassis commersoni* was treated with 3 different concentrations (0·1, 0·25 and 0·5 ppm) of cadmium upto 120 days. Fishes treated in higher concentrations developed vertebral deformity and the deformation was prominent after 89th and 115th day. The levels of the vertebral minerals such as calcium, magnesium and phosphorus lowered in all exposure concentrations and minerals lost were directly proportional to the toxic concentrations. Nearly 2/3 of skeletal mineral composition was eliminated in the deformed fish. Vertebral fracture was attributed mainly to the large amount of vertebral components eliminated from the vertebrae. Vertebral cadmium accumulation was also linear in relation to the experimental concentrations.

Keywords. Cadmium; caudal curvature; vertebral deformities; calcium; phosphorus; magnesium; elimination.

1. Introduction

The toxic effects of cadmium (Cd) on fishes are known (Eisler 1971; Selvakumar 1981) and it has been identified as a causative factor for the outbreak of 'Itai-itai' disease in Japan. Pathological changes (Gardner and Yevich 1970) and depression of carbohydrate metabolism (Larsson 1975; Larsson and Haux 1982) and oxygen consumption (Selvakumar 1981) by Cd have also been reported. Exposure to Cd for longer durations are also known to induce vertebral deformities in fishes (Bengtsson *et al* 1975). Reports on skeletal deformities induced by chemical agents are very limited. Weis and Weis (1976) and Couch *et al* (1977, 1979) recorded pesticide-induced vertebral deformities in *Cyprinodon variegatus*. Linden (1976) noted that crude oil also caused vertebral malformation in *Clupea harengus membras* within a short period of exposure.

Bengtsson (1974) and Holcombe *et al* (1976) noted vertebral malformations in zinc-treated *Phoxinus phoxinus* and lead treated *Salvelinus fontinalis* after 4 months and 67 weeks of exposure respectively.

The present investigation was carried out on an estuarine fish *Ambassis commersoni* Cuvier by exposing them to 3 different sublethal Cd concentrations for 120 days. At the close of the experimental period, the Cd content and the mineral composition of the Cd treated fish vertebrae were also estimated.

2. Materials and methods

Specimens of *A. commersoni* were collected from the marine zone of the Vellar estuary. The length of the fishes used was $6{\cdot}3 \pm 0{\cdot}2$ cm and the weight $2{\cdot}5 \pm 0{\cdot}5$ g. The

fishes were kept in acclimatisation tanks for a week in the laboratory. The salinity of the test water ranged between 27 and 28‰. Fishes were fed regularly with chopped clam meat. Healthy fishes were selected and exposed to Cd concentrations of 0·1, 0·25 and 0·5 ppm (each set consisted of 12 specimens). These exposure concentrations were prepared on the basis of 96 h LC_{50} value (5·20 ppm). Cd solution was prepared from Analar $CdCl_2 \cdot H_2O$ salt. The experimental fishes were fed every day during all the 120 days.

After 120 days of experimental exposure period, the fishes were anaesthetized with MS 222 and the vertebral damages if any were located by X-ray photograph using a Siemen-160 (West Germany) X-ray equipment. The vertebral Cd concentration was estimated by the method of Smith and Windom (1972) using a Hilger-Watts Atmospek H 1550 AAS.

The vertebral column of the untreated and treated fishes were dried at 60°C for 24 h in a oven. The dried samples were ashed by heating them for 12 h at 500°C in a muffle furnace. The ashes were then dissolved in 2:1 of concentrated HNO_3 + concentrated $HClO_4$ and made upto 100 ml with double distilled water. The vertebral calcium (Ca) and magnesium (Mg) levels were estimated following the method of Katz and Narone (1964). Phosphorus (P) was estimated by the procedure of Strickland and Parsons (1972) and concentrations were noted in a Hitachi double beam UV spectrophotometer Model 220.

3. Observations

The concentration of Cd as well as those of Ca, Mg and P in the vertebrae of both untreated and treated fishes were estimated (figure 1).

Two fishes were observed with vertebral abnormalities out of 12 fishes exposed to 0·5 ppm Cd concentration. Caudal vertebral curvature (figure 2B) was clearly observed in one fish after 89 days of Cd exposure. After 115 days, another fish in the same experimental tank with abnormal swimming movement was noted. The damage (figure 2C) was located in the vertebral column through x-ray photograph in this fish with abnormal swimming movement.

The Cd content of the vertebrae in the control fishes revealed no traces of Cd. However in fishes exposed to Cd, its accumulation in vertebrae was seen. After 120 days of exposure the vertebral Cd concentrations were 8·32, 10·91 and 12·86 μg/g in respect of the 3 exposure concentrations, viz 0·1, 0·25 and 0·5 ppm (figure 1).

In the untreated fishes the vertebral mineral levels were found to be in the following order: (i) Ca 132·81 mg/g, (ii) P 52·97 mg/g and (iii) Mg 3·27 mg/g. After the experimental period the mineral levels were low in all Cd treated fishes and showed the following pattern: (i) Ca 120·72, 116·36 and 106·41 mg/g, (ii) P 46·59, 46·50 and 34·86 mg/g and (iii) Mg 3·08, 2·82 and 2·16 mg/g respectively in the 0·1, 0·25 and 0·5 ppm of Cd concentrations to which they were exposed. It is evident from the results that the loss of minerals from vertebrae was dose dependent (figure 1).

The Cd content (18·97 μg/g) in vertebrae of abnormal fish was higher than that in other treated fishes. Nearly 58% of Ca, 56% of P and 67% of Mg were lost from the vertebrae of the deformed fish. The skeletal mineral composition was found in the following order: (i) Ca 56·13 mg/g, (ii) P 23·49 mg/g and (iii) Mg 1·09 mg/g.

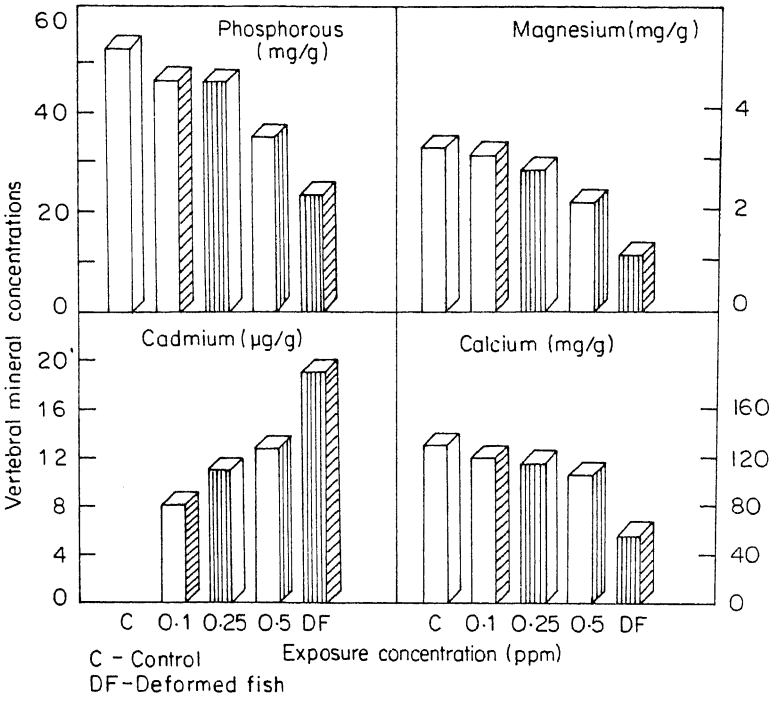

Figure 1. Concentrations of Cd, Ca, Mg and P in the vertebrae of both untreated and treated fishes.

3. Discussion

In the present investigation vertebral deformities were observed in treated fishes only after the 89th day (caudal curvature) and 115th day of exposures (with vertebral damage) at a Cd concentration of 0·5 ppm (figure 2). Eaton (1974), Bengtsson *et al* (1975) and Muramoto (1981a, b) also recorded similar vertebral anomalies in Cd treated *Lepomis macrochirus*, *Phoxinus phoxinus* and *Cyprinus carpio* L. respectively. From the results obtained in this study it is apparent that more than 2/3 of the vertebral Ca, Mg and P were found to be eliminated in deformed fishes. These were lowered to a considerable extent in all Cd treated normal fishes. The results of the present study corroborate the findings of Muramoto (1981a, b) on *Cyprinus carpio* L.

The caudal curvature and vertebral damages in *A. commersoni* might have been influenced by the loss of Ca, P and Mg from the vertebral column. The work of Matsunaga *et al* (1962) provides evidence to show the occurrence of as many as 72 fractures in 'Itai-itai' patients affected by Cd food poisoning, which led to large scale elimination of minerals from the bones. Koyama and Itazawa (1977a, b) and Itokawa *et al* (1978) also noted vertebral deformation, vertebral mineral elimination and skeletal lesions in Cd exposed *Cyprinus carpio* L. and rat. In the present study, abnormal swimming behaviour, altered movement and flexibility of body of fish might have resulted from excess elimination of skeletal mineral like Ca, P and Mg.

Larsson (1975) and Larsson *et al* (1981) have suggested that the vertebral demineralization in Cd-treated fishes might be mainly due to imbalance in Ca

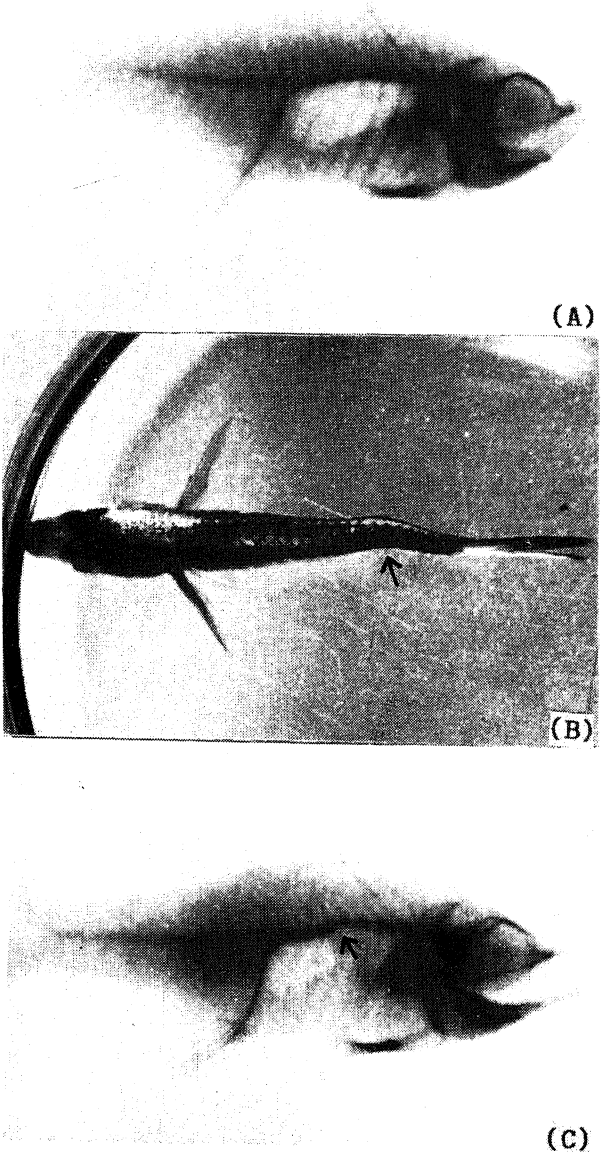

Figure 2. **A.** Normal fish. **B.** Vertebral anomalies with caudal curvature. **C.** Vertebral deformed fish.

metabolism controlled by endocrine glands. In the present study, during Cd exposure, the excess (body-accumulated) Cd ions might have affected the Ca and P metabolism of thyroid and parathyroid functions which in turn led to vertebral mineral elimination. This aspect needs further study.

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Distribution and population dynamics of soil nematodes in a tropical forest ecosystem from Sambalpur, India

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Abstract. An ecological study on soil nematodes was made in a tropical deciduous forest ecosystem. Seventeen species of nematodes were identified of which *Rotylenchus* sp. was the dominant plant parasitic form and *Acrobeloides* sp. was the dominant microbivore species. The mean annual importance value for the miscellaneous feeders, plant parasites, microbivores and predators were 57·5, 22·5, 12·6 and 7·4 respectively. The Shannon index of general diversity was maximum during February (1·73) and minimum during June (1·41). Nematodes were distributed in clusters resulting in so called pocket effect. Of the total nematodes 88·4% occurred in the top 10 cm soil during the peak period of density and the microbivores were more frequent in the top 5 cm soil due to the litter layer. Total nematode density ranged from $15{\cdot}1 \times 10^4/m^2$ (May) to $66{\cdot}1 \times 10^4/m^2$ (November). Monthly mean nematode biomass was $18{\cdot}86 \pm 8{\cdot}36$ mg dry wt/m^2. Temperature, soil organic carbon and soil total nitrogen apparently played an important role in regulating the nematode population.

Keywords. Soil nematode; forest ecosystem; importance value; diversity; organic carbon; nitrogen.

1. Introduction

Nematodes are an important consumer group in the decomposer subsystem of many ecosystems. Wasilewska (1970, 1971) in Poland, Yeates (1972, 1973) in Denmark and Johnson *et al* (1972, 1973, 1974) in USA have studied the soil nematodes in the forest ecosystems. However, knowledge on the ecology of soil nematodes from the forest ecosystems of India is non-existent. The results presented here on the diversity, distribution, population dynamics and biomass of soil nematodes of a tropical forest ecosystem in relation with climatic and soil factors are the first of its nature from India.

2. Study site

The study site forms part of Ushakothi National Wild Life Sanctuary located 60 km north-east of Sambalpur University Campus. The dominant tree in the forest site was *Shorea robusta* Gaertn. and during the rainy season there was a thick growth of seasonal plants like *Croton sparsiflorus* Morung., *Euphorbia hirta* L. and *Hygropilla spinosa* T. Anders etc. The soil was sandy clay loam and the pH was 5·82 in the top 10 cm soil.

The area experiences 3 seasons (rainy, winter and summer). The annual rainfall being 1290 mm of which 88% rain fell during the rainy season. The minimum air temperature varied from 10°C (December) to 45°C (May). The average relative

humidity was minimum during April (46%) and maximum during August (84%). The soil moisture ranged from 3·5% (May) to 18·8% (August).

3. Methods

At the study site a plot of 121 m^2 in area was taken. Mostly samples were collected from December 1980 to March 1982. Each month 7 sample units were taken and divided into 5 subsamples: 0–5, 5–10, 10–15, 15–20 and 20–30 cm soil layers. The samples, each 10 cm^2 in area × 30 cm deep, were selected at random on a 1 × 1 m grid.

For extraction of soil nematodes, about 250 cm^3 of soil for each subsample was processed by Cobb's (1918) wet screening and then by modified Baermann funnel technique (Southey 1970). Senapati and Dash (1976) had observed that 90% of the total soil nematodes were recovered by this method. Mishra and Dash (1981) also had followed this method while studying the soil nematodes of the rice field and pasture of Sambalpur. Samples were kept for 24 h on the funnel. Nematodes recovered were classified into 4 ecological feeding groups (Banage 1963): (i) Plant parasites (PP), (ii) microbivores (MR), (iii) miscellaneous feeders (MS) comprising Dorylaimida-food varied and largely unknown, and (iv) predators (PR) mostly Mononchidae. Each month, after counting, the nematodes were fixed with hot FA 4:10. For the identification and biomass estimation permanent mounts were made from the fixed nematodes by glycerol ethanol method (Southey 1970). Total numbers of all the trophic groups/m^2 were calculated by taking all the subsamples of each layer into account. Maximum body width and length were measured for the biomass calculation as per Andrassy (1956). To assess the relationship of soil nematodes with soil nutrients, the total organic matter and total nitrogen content of all the soil samples were estimated respectively by muffle furnace method (Paine 1971; Reiners and Reiners 1972) and micro-kjeldahl method (Jackson 1973).

4. Results

4.1 *Diversity*

Table 1 shows the species diversity of the study site. *Rotylenchus* sp. was the dominant plant parasitic form found in every sample. *Hoplolaimus seinhorsti* Luc and *Hemicycliophora oostenbrinki* Luc were sometimes found in deeper soil layers in the rainy and winter seasons. Other plant parasitic species were seen occasionally.

The microbivores were very large in number and *Acrobeloides* sp. was dominant occurring in every sample decreasing with depth. Other microbivore species were mainly found in the upper soil layers. The miscellaneous feeders were abundant throughout the year and 6 species were identified. The predators were very few in number.

The importance value (IV) of the nematode trophic groups were calculated by the formula;

$$IV = \frac{Wa}{Wt} \times 100,$$

Table 1. Species diversity in the study site.

Nematode trophic group	
Plant parasitic forms	*Rotylenchus* sp.
	Hemicycliophora oostenbrinki Luc
	Hemicriconemoides cocophilus (Loos) Chitwood and Birchfield
	Hoplolaimus seinhorstii Luc
	Orientylus sp.
Microbivores	*Acrobeloides* sp.
	Cephalobus sp.
	Acrobeles sp.
Miscellaneous feeders	*Alimus* sp.
	Dorylaimus sp.
	Eudorylaimus sp.
	Aporcelaimus sp.
Predators	*Mylonchulus mulveyi* Jairajpuri
	Mononchus dentatatus Cobb
	Coomansus indicus Jairajpuri and Khan
	Mononchus papillatus Bastian
	Nygolaimus sp.

where Wa is the dry weight of a trophic group and Wt is the dry weight of all the trophic groups in the sampling plot.

The mean annual importance values of the miscellaneous feeders, plant parasites, microbivores and predators were 57·5, 22·5, 12·6 and 7·4 respectively.

The index of general diversity ($\bar{H}$) for nematodes were computed (Shannon and Weaver 1949). The index of diversity ranged from 1·41 (June) to 1·73 (February) indicating peak diversity in fauna in February.

4.2 *Distribution*

The cluster analysis (Norton 1978) during the period of maximum and minimum population density from various sampling plots showed that nematodes were aggregated than distributed uniformly or randomly (figure 1).

The plant parasites were abundant in the 0–15 cm layer only in winter. The microbivores were most abundant in the 0–5 cm soil layer and less in number below 5 cm throughout the year. The miscellaneous feeders were larger in size but decreased in number with the increase of depth or season.

The nematode population per m^2 for each month and for each soil layer are shown in figure 2. The nematode population ranged from 7·3 to $44{\cdot}4 \times 10^4/m^2$ in 0–5 cm soil layer, 3·8 to $14{\cdot}4 \times 10^4/m^2$ in 5–10 cm soil layer, 1·5 to $7{\cdot}2 \times 10^4/m^2$ in 10–15 cm soil layer, 0·8 to $4{\cdot}1 \times 10^4/m^2$ in 15–20 cm soil layer and 0·6 to $3{\cdot}7 \times 10^4/m^2$ in 20–30 cm soil layer. This indicated the decrease of total nematode numbers with increase of soil depth. The nematodes were mainly distributed in the upper 10 cm soil layer in all the seasons being 88 and 81% of the total nematodes during the maximum (November) and minimum (May) density of the year respectively. The mean annual nematode density was 3·6 times more in the 0–10 cm soil layer than the 10–30 cm soil layer.

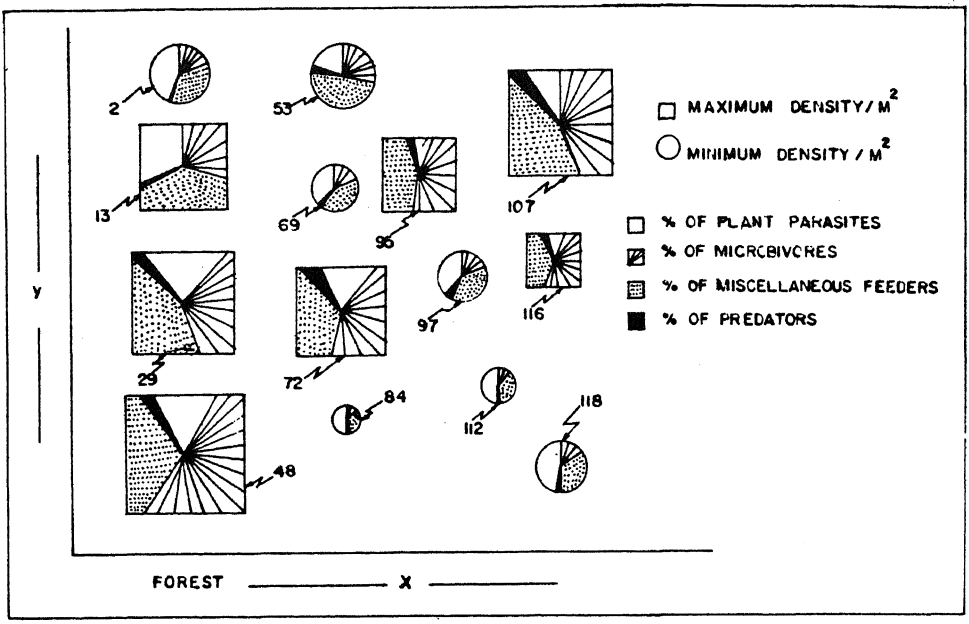

Figure 1. Ordination of 4 trophic groups of nematodes during maximum and minimum population density. Squares and circle sizes depict abundance in study sites.

The total nematode density ranged from $15{\cdot}1 \times 10^4/m^2$ (May) to $66{\cdot}1 \times 10^4/m^2$ (November) during the study period. The population density increased gradually during rainy season reaching the peak density in winter and then declined gradually reaching minimum density in summer.

4.3 *Biomass*

For the determination of biomass, measurement of greatest body length and body width of 15 numbers of nematodes of each trophic group from different soil layers and different seasons were done. The average dry weights (μg) for plant parasite, microbivore, miscellaneous feeder and predator were 0·05, 0·02, 0·06 and 0·12 respectively. The average individual dry weight was 0·07 μg. The nematode dry weight biomass ranged from 9·6 mg/m^2 (May) to 37·0 mg/m^2 (November) with a monthly mean of $18{\cdot}86 \pm 8{\cdot}36$ mg/m^2. The maximum to minimum biomass ratio and maximum to average biomass ratio comes to about 4 and 2.

4.4 *Soil nutrients*

The organic carbon (4·2%) and total nitrogen (0·26%) increased after the commencement of rain in November and then gradually declined and reached minimum in May (3·3% and 0·1%). The nematode biomass showed a positive correlation with soil organic carbon ($r = +0{\cdot}53$) and soil total nitrogen ($r = +0{\cdot}64$) during sampling months (figure 3).

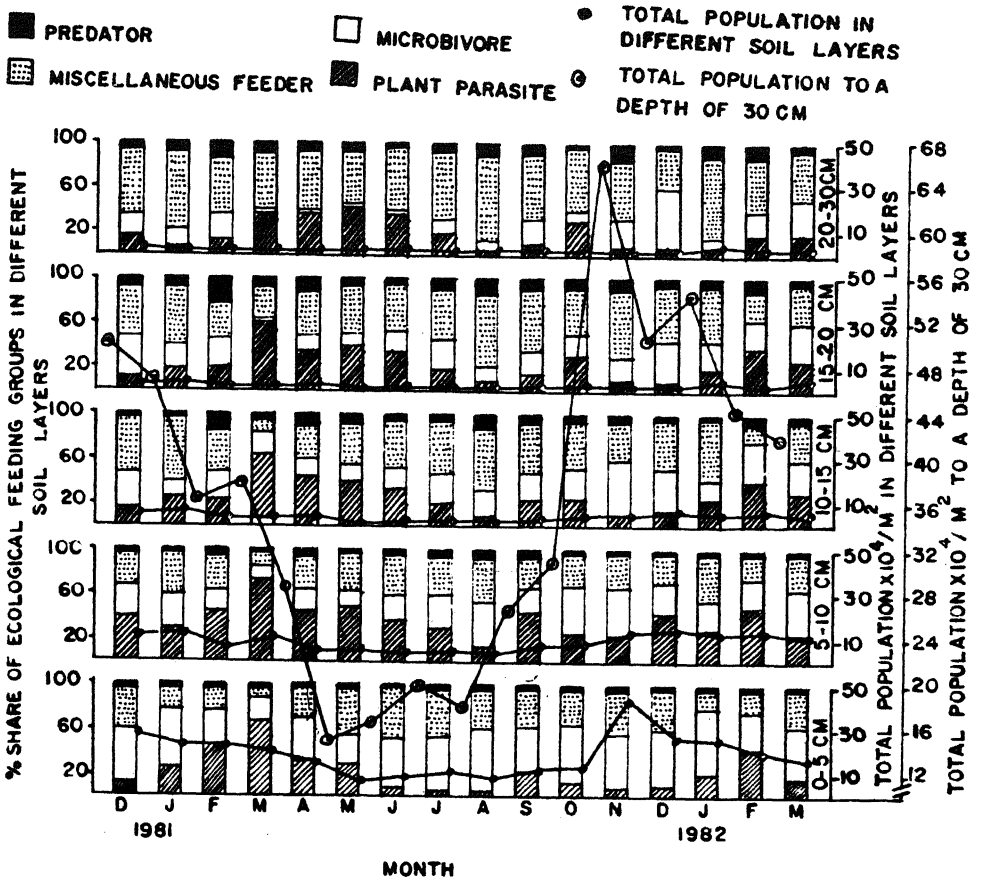
PREDATOR
MICROBIVORE
MISCELLANEOUS FEEDER
PLANT PARASITE
TOTAL POPULATION IN DIFFERENT SOIL LAYERS
TOTAL POPULATION TO A DEPTH OF 30 CM
% SHARE OF ECOLOGICAL FEEDING GROUPS IN DIFFERENT SOIL LAYERS
TOTAL POPULATION X10⁴/M² IN DIFFERENT SOIL LAYERS
TOTAL POPULATION X10⁴/M² TO A DEPTH OF 30 CM
20-30CM
15-20 CM
10-15 CM
5-10 CM
0-5 CM
D J F M A M J J A S O N D J F M
1981
1982
MONTH

Figure 2. Population dynamics, biomass and per cent share of ecological feeding groups of nematodes in different soil layers of the study site.

5. Discussion

5.1 *Diversity*

The greater the fluctuations in species diversity of a community, the less is its stability (Mac Arthur 1955). The diversity index ranging from 1·41–1·73 does not show heavy fluctuations and indicates a stable community. In the forest, nematodes are mostly abundant in the decomposition zone, where there is greater microbial activity (Twinn 1974). The present finding is in agreement with Twinn's observation as the nematodes were comparatively more in the upper 0–10 cm layer containing high organic matter. This has also resulted in higher number of microbivores and miscellaneous feeders in the upper layer. *Shorea robusta* was the dominant tree of the study site. The less diversified understorey vegetation and accumulation of organic matter might have resulted in the occurrence of fewer plant parasitic species.

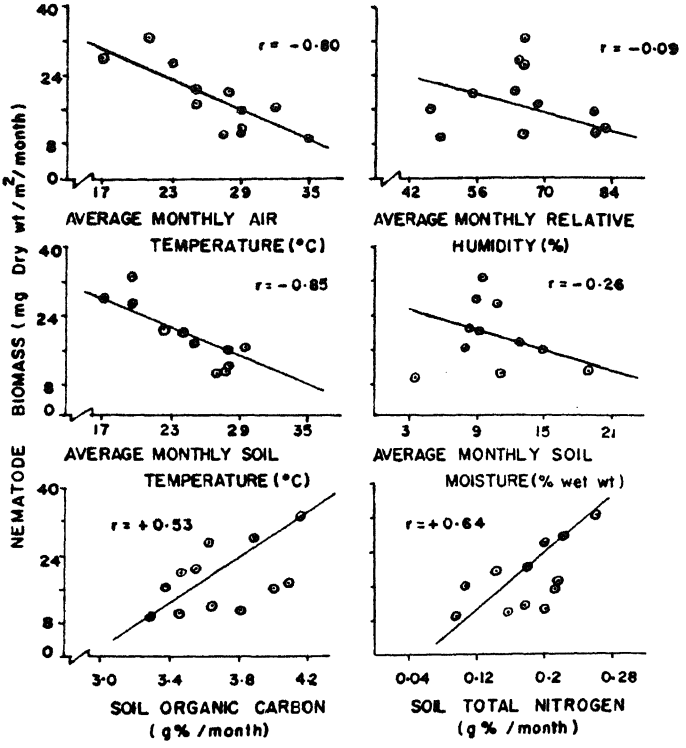
NEMATODE BIOMASS (mg Dry wt / m² / month)
r = -0·80
AVERAGE MONTHLY AIR TEMPERATURE (°C)
r = -0·09
AVERAGE MONTHLY RELATIVE HUMIDITY (%)
r = -0·85
AVERAGE MONTHLY SOIL TEMPERATURE (°C)
r = -0·26
AVERAGE MONTHLY SOIL MOISTURE (% wet wt)
r = +0·53
SOIL ORGANIC CARBON (g% / month)
r = +0·64
SOIL TOTAL NITROGEN (g% / month)

Figure 3. Correlation between environmental factors, soil nutrients and total nematode biomass of the study site.

5.2 *Density*

Nematode population densities varied from site to site. Based on the data of Petorson, Yeates (1979) had tabulated the average nematode population which was 3,900,000/m^2 for tundra ecosystem, 1,919,000/m^2 for 6 temperate grasslands, 423,000/m^2 for a desert, 2,811,000/m^2 for 9 deciduous forests and 56,000/m^2 for a tropical forest. Population estimates ranged from 50,300 to 312,800/m^2 and 40,000 to 215,900/m^2 in the tropical rice field and pasture soil of India (Mishra and Dash 1981). In this investigation the population density ranged from 150,600 to 661,100/m^2 which was more than other above reported tropical soils but very less when compared with other temperate soils. The ratio between maximum and minimum nematode density was around 4 and it was within the range reported in previous findings (Yeates 1979).

Previous studies indicate that changes in soil moisture and temperature are related with population changes. The decline in nematode abundance from November to May followed the increase in temperature and was correlated with a very dry period. During rainy season with increased soil moisture and moderate temperature recruitment to the fauna continued and the nematode population density reached peak in November when the temperature was low and soil moisture was moderate. Nematode biomass showed a significant negative correlation with soil temperature ($r = -0{\cdot}85$,

$n=12$, $P<0{\cdot}01$) and air temperature ($r=-0{\cdot}80$, $n=12$, $P<0{\cdot}01$). This was in conformity with the findings of Mishra and Dash (1981). The nematode biomass was negatively correlated with relative humidity (%) and soil moisture (figure 3).

5.3 *Biomass*

The mean individual nematode dry weight of this study site was comparable with other temperate forest ecosystems (Yeates 1979) but very less than the tropical rice field and pasture of India (Mishra and Dash 1981). The proportionately higher number of microbivores with lower individual dry weight might have resulted in relatively lower biomass value in relation to population size for tropical soils.

5.4 *Nutrients*

Stockli (1952) suggested that nematode abundance is linked with readily decomposing organic substrate. The nematode abundance was dependent on the litter biomass (Dash and Pradhan 1984) due to greater variety of food source available, high organic matter, higher fungal biomass (Behera and Dash 1980) and bacterial biomass. The organic matter is important in nematode ecology (Norton 1978) and significant positive correlation exists between soil nematodes and soil organic carbon (%) (Yeates 1979). In the forest site the decomposing litter layer has resulted in altering the soil characteristic features like organic carbon and nitrogen, both of which are related to plant growth and essential for the growth of soil organisms.

Acknowledgements

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Biology of *Nyphasia apicalis* Gahan (Coleoptera: Cerambycidae) with particular emphasis on emergence and imaginal life

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Abstract. Biology of the sapwood borer, *Nyphasia apicalis* Gahan is discussed with special reference to some aspects of adult behaviour. Development and survival of eggs and pupae are dependent on high humidity. Life-cycle is annual but may be prolonged to the next year. Adult emergence mainly occurs in June and July and is synchronized with the onset of rains. Beetles are diurnal, being most active in bright sunlight and are flower visitors feeding on different floral components. They are sexually mature on emergence and mate soon afterwards usually on the material from which they have emerged. Females prefer logs after a month of felling for oviposition. Logs lying in shady areas are avoided by the ovipositing females.

Keywords. *Nyphasia apicalis*; Cerambycidae; overwintering; adult emergence; emergence stimulus; adult behaviour.

1. Introduction

Depredation of longicorn beetles almost essentially demands an overall assessment of their damage potential along with their ecobiological characteristics. Any investigation directed towards their imaginal life would appear equally important to save the forest and forest products from their damage. However, we still have only a marginal knowledge of these important phenomena, although we have been suffering from a serious Cerambycid damage for decades, costing several millions of rupees per year (Beeson 1941).

Keeping these in view, work has been undertaken to study the ecobiology and damage potential of this group of beetles. Present communication summarises the biology of a sapwood borer, *Nyphasia apicalis* Gahan, with special reference to its emergence and imaginal life. The species is fairly common in India and breeds in 3 economically important timber yielding plants, viz *Anogeissus acuminata*, *Shorea robusta* and *Terminalia tomentosa*. Available biological information of this borer is limited to the above mentioned host-plants, duration of life-cycle, emergence period and some aspects of the adult behaviour (Beeson and Bhatia 1939).

2. Material and methods

2.1 *Field observations*

Field work was carried out during different months, particularly from June–August of 1982–1984 in forest areas and timber depots in Midnapur District, West Bengal

and in Singhbhum District, Bihar, to collect data on life-cycle, behaviour of immature stages, adult emergence, flight, feeding, mating and other relevant activities. Detailed observations on the emergence were, however, made from infested logs taken in galvanized iron cages. The number of adults emerged on subsequent days was determined from emergence-holes on the bark surface. The emergence-holes of each day were marked so as to detect new ones.

Mating and oviposition were studied using freshly felled bait trees. These trees were cut into billets of uniform size. Time spent in courtship and copulation was determined from direct observation on individual pairs and by comparing this with the time utilized in other activities. Adults visiting the bait logs were captured, marked with white paint on elytra and released. The number of marked adults that visited the bait logs and the number oviposited thereon on subsequent days were recorded. For each day, the egg-niches made by females on each of the logs were also counted and marked with paints. Five marked sample females were collected on alternate days of observation and held in breeding-cages for further oviposition in the field.

Host condition preference studies were made with Sal (*Shorea robusta*) logs of different period lapse after felling. The oviposition data obtained from the bait Sal billets were also utilized for this purpose. The infested logs were examined carefully and dissected for the life-cycle stages of the borer. Presence of eggs and early instar larvae was taken as the indicator of fresh infestation.

2.2 *Laboratory observations*

Laboratory studies were conducted in Calcutta using portions of infested billets and branches (30–45 cm in dia) of the host-plants collected from different field sites. They were held in galvanized iron cages of 1 m × 50 cm × 50 cm (Khan 1985) for adult emergence. Adult beetles that emerged were collected between 0900 and 1000 hours daily. They were counted, sexed and were reared in glass breeding-cages (Khan and Maiti 1982a) for behavioural observations.

Oviposition and fecundity were observed with newly emerged adults (Khan and Maiti 1982b). Twenty five males and females each were separately kept in breeding-cages. They were provided with different floral parts of the larval host-plants as food and billets of Sal after 3 weeks of cut (25–30 cm long and 8–12 cm in dia) for oviposition, both of which were replaced on alternate days. The number of oviposition slits on the billets was counted and the number of eggs laid thereon was recorded.

Approximate duration of egg and pupal stage were estimated under simulated field conditions, where an attempt was made to simulate the field conditions following Saliba (1977). The effects of moisture on the development of egg was determined by placing each of the 3 batches of 100 eggs, first in petridishes containing moist filter paper, while the second containing dry filter paper. Petridishes of both the sets were separately kept in air-tight desiccators each containing a hare hygrometer inside it. The humidity ranged from 75–90% and from 42–66% with moist and dry filter paper. The third batch of eggs was kept in open petridishes exposed to sunlight (with about 6 h daily exposure). All petridishes were examined and the number of eggs hatched was recorded daily. Eggs which failed to hatch within a period of 3 months were taken as dead.

Larval development was studied following Khan and Maiti (1982a). Larval instars were determined from head-capsule measurements. The exuviae, particularly the head-capsule of the earlier instar was also considered as an indicator of moult, as it offers a clue for the same (Khan and Maiti 1983).

Studies on the development and survival of pupae were carried out with mature larvae which had ceased feeding. They were separately kept in glass tubes containing moist or dry filter paper at the bottom. All other procedures were similar to those of the studies with eggs.

3. Results

3.1 *Development and behaviour of immature stages*

3.1a *The egg and its development*: Eggs are white, oval, 1·7 mm long and 0·7 mm broad. They are usually laid in groups of 2–10 in cracks and crevices in the bark or on the under surface of the bark through cut edges of logs. The approximate incubation period varied from 12–16 days. Results obtained from the studies with dry and moist filter paper (table 1) clearly indicate that humidity plays an important role in the development of eggs, particularly regarding their viability. Total mortality of eggs occurred in direct sunlight.

3.1b *Larval development*: Newly hatched larvae, after a brief period of feeding on the empty egg-shell, bore through the bark to reach the subcortical tissue and excavate tiny galleries radiating on the wood surface in all directions from the oviposition site. The first moult normally takes place after about a fortnight of eclosion. Second-instar larvae feed vigorously on the subcortex as they tunnel through it. This phase lasts for about a month. Other larval instars are not so clearly defined. Larval tunnels remain mainly confined to the region between the bark and phloem-cambium layer of the wood. These are oval in cross section and are packed tightly with a powdery frass (figure 1a).

Larval life extends for a varying period from about 7–12 months depending on the season and the size of the host. An excessively long larval life is invariably associated with overwintering. Thus, larvae develop during the post-monsoon period overwinter and complete their larval life during the pre-monsoon period of the next year

Table 1. Viability and incubation of eggs of *N. apicalis* under different conditions.

	No. of eggs	No. of eggs hatched after oviposition (days)							Total hatch	Hatch (%)
		12	13	14	15	16	17	18		
Simulated field	169	43	46	31	11	6	0	0	137	82
Moist filter paper	100	38	31	9	5	0	0	0	83	83*
Dry filter paper	100	0	14	17	8	6	2	2	49	49**
Direct sunlight	100	0	0	0	0	0	0	0	0	0

*$P<0.01$; **$P<0.001$; the difference between the moist and dry filter paper is significant at 0·1% level.

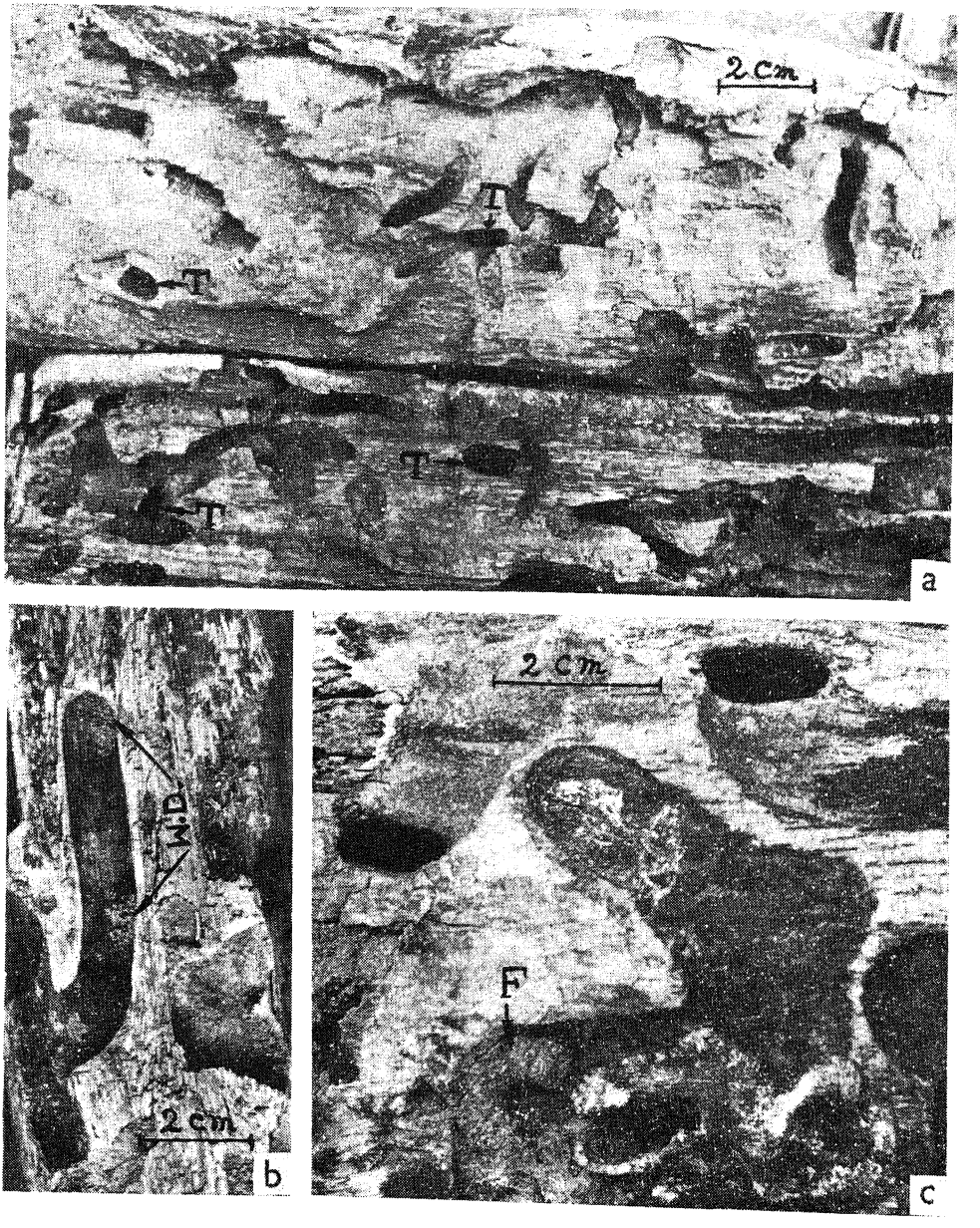

Figure 1. **a.** Larval tunnels of *N. apicalis* inside the sapwood of *T. tomentosa*. The powdery frass packing the tunnels and appearance of a few tunnels (T) in cross section are noticeable. **b.** Pupal chamber of *N. apicalis* inside the sapwood of *T. tomentosa*. Proximal and distal end of the chamber are found filled with wood-dust (WD). **c.** The roughly oval exit-holes of *N. apicalis* on the wood-surface of *T. tomentosa*. Fine powdery frass (F) is visible inside a larval gallery.

or later. Present observations suggest that overwintering may be associated with any stage of larval life, since a considerable number of overwintering larvae belonging to different developmental stages have been collected during the present study. The

shortest larval life was observed among the larvae developing during the period from June–October.

3.1c *Pupation and pre-emergence resting*: Shortly after the last moult, the larvae start mining in the wood. However, a small percentage of the larvae, particularly in smaller logs, may bore inside the wood much earlier. The pupal chamber normally varies from 2·19–3·76 cm in length and from 0·78–1·03 cm in width. They are usually located at a depth of 2·6–6·8 cm inside the wood. Proximal and distal ends of these chambers are filled tightly with fine wood-dust (figure 1b). Before filling the proximal end of the pupal chamber, the larva widens and cleans the pre-pupal tunnel for future adult emergence.

The pupal period is 3 to 4 weeks under the simulated field conditions. The development and survival of pupae greatly depend on humidity like eggs. Thus, 80% of the pupae maintained in moist condition yielded adults and the pupal period was 20–26 days, while those in dry condition took 24–32 days with only 48% viability (table 2). Pupae exposed to direct sunlight (as in eggs) failed to develop into adults.

Under field conditions, however, the duration of the pupal stage varies considerably. Field observations made during the period from September 4 to November 20, 1983, revealed first pupa, first dormant adult and first emerged adult on September 18, October 9 and October 30, respectively (table 3). These observations suggest a pupal period and pre-emergence resting period of about 3 weeks each.

Both the pupal and adult dormancy period vary considerably depending on the season and the overwintering pupae and dormant adults require a longer period to complete their developmental phase than the others.

3.1d *Developmental period*: The species is known to have an annual life-cycle, which may be prolonged to the second year in adverse conditions (Beeson and Bhatia 1939). It has been observed on a number of occasions that duration of life-cycle in individuals of even the same progeny varies widely. Developmental period is prolonged due to overwintering. In general, eggs laid during pre-monsoon period require 8–9 months to develop into dormant adults which overwinter and emerge during the next pre-monsoon. In contrast, those laid during the post-monsoon period or later, overwinter as larvae and complete their development during the late monsoon period of the next year or later, although a small percentage of the progeny

Table 2. Development and survival of pupae of *N. apicalis* under different conditions.

	No. of pupae	Adult emergence after pupation (days)							No. of adults emerged	Survival (%)
		20	22	24	26	28	30	32		
Simulated field	50	6	11	11	7	3	0	0	38	76
Moist filter paper	50	11	19	6	4	0	0	0	40	80*
Dry filter paper	50	0	0	3	6	7	4	4	24	48**
Direct sunlight	25	0	0	0	0	0	0	0	0	0

*$P<0·01$; **$P<0·001$; the difference between the moist and dry filter paper is significant at 0·1% level.

Table 3. Distribution of larva, pupa, dormant adult and adult of a cohort of *N. apicalis* developing in Sal (*S. robusta*) during post-monsoon period.

Date of observation		Frequency distribution (%) Larva	Pupa	Dormant adult	Adult	Total No. of insects
Sept.	4	100·0	0	0	0	109
	11	100·0	0	0	0	113
	18	96·0	4·0	0	0	124
	25	86·4	13·6	0	0	110
Oct.	2	78·0	22·0	0	0	150
	9	50·6	45·9	3·5	0	180
	16	38·0	51·0	11·0	0	163
	23	26·7	44·4	28·9	0	135
	30	17·8	37·2	41·3	3·7	191
Nov.	6	11·9	26·9	50·3	10·9	193
	13	8·0	19·9	43·8	28·3	201
	20	5·0	16·8	37·4	40·8	179
					Total	1,848

particularly in smaller and drier material may not be able to complete their development during this period. They are, therefore, forced to overwinter again to emerge during the next pre-monsoon or later.

However, on the basis of the present observations, as discussed in the foregoing chapters, the duration of different developmental stages and the total developmental period of this species may be summarized as follows:

Egg	2–3	weeks
Larva	30–52	»
Pupa	3–9	»
Dormant adult	3–11	»
Total	38–75	»

The maximum and minimum developmental period is based on maximum and minimum duration of different developmental stages and is not observed practically. This is due to the fact that one or the other developmental stage normally extends to more than the minimum duration.

3.2 *Adult emergence*

The species does not excavate any special exit-tunnel for adult emergence and the emerging adults exit by utilizing the pre-pupal tunnel as much as possible and finally gnawing through the bark. Exit-holes are roughly oval (figure 1c).

N. apicalis is known to have a pre-monsoon emergence during May and June extending up to July in North India, with peak emergences sometime during the last week of May and the second week of June (Beeson and Bhatia 1939). However, the present data (table 4) indicate that the species emerges mainly during June and July in this South-eastern area of India. Moreover, the date of the commencement of emergence varies from year to year synchronizing with the date of the first heavy

Table 4. Field collection data (excluding those collected from intensive study sites) of *N. apicalis* during 1982–1984.

Year	Date of first heavy showers in the area	Date of first appearance of adults	Number of beetles collected during different months							
			J to A	M	J	J	A	S	O	N and D
1982	June 17	June 18	0	0	54	42	25	10	3	0
1983	May 27	May 29	0	29	47	55	31	11	4	0
1984	May 23	May 24	0	38	58	63	28	9	2	0

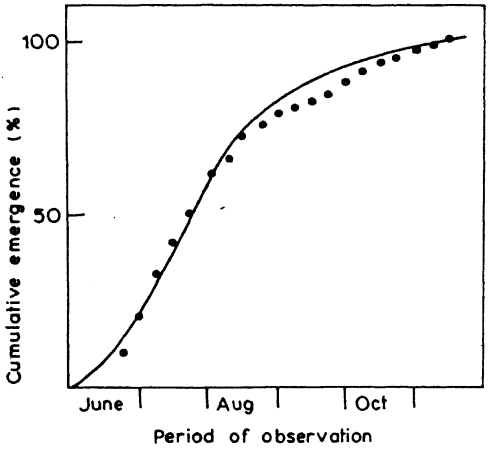

Figure 2. Insectary emergence of a cohort of *N. apicalis* from a portion of a Sal (*S. robusta*) log. The cohort was consisting of 149 individuals.

showers of rains. Once emergence starts, it may extend even up to the middle of September.

In the insectary, on the other hand, emergence period extended from mid-June to mid-November. Emergence was invariably initiated one or two days after heavy showers of rains, although the insectary was maintained unexposed to natural conditions. Figure 2 presents the emergence data of a cohort, where emergence was initiated on June 21 following heavy rains on the afternoon and night of June 20. There were atleast 15 heavy showers of rains, mostly from June to mid-August, during the emergence period of this cohort. It is remarkable that about 10% of the adults emerged during a period with no rains from the second week of October to mid-November. This was true for other caged cohorts also.

Foregoing observations may indicate a possible synchronization between the onset of rains and adult emergence, as it is true for another common species of the area, the well known Singhbhum Sal borer, *Hoplocerambyx spinicornis* (Newm.) (Beeson and Bhatia 1939). This was studied during the emergence period of the species (June and July) in some timber depots with infested Sal (*Shorea robusta*) logs. At the beginning of the study, adults in the pupal chambers were mature and capable of flying away as the wood was split. Results for 1982 are presented in figure 3a, where the amount of rainfall is indicated in terms of light, moderate and heavy and adult emergence is compared with relative humidity. Emergence-holes on logs

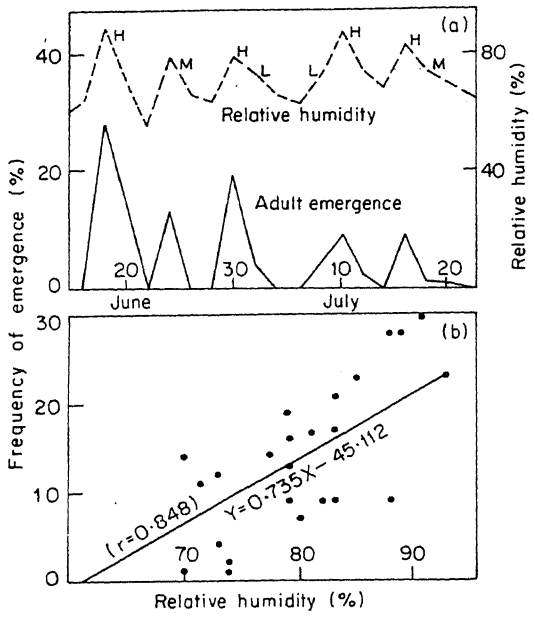

Figure 3. Relationship between relative humidity, rainfall and adult emergence of *N. apicalis*. (**a**) The emergence and humidity pattern and rainfall during the emergence period of 1982 (L, light; M, moderate and H, heavy rains). (**b**) Curvilinear relationship between the relative humidity and adult emergence ($P < 0{\cdot}001$).

appeared, as well as, the first adult was taken at lights on June 18, following the first heavy showers of rains in the area on June 17. Average relative humidity during those two days was 89%. The emergence pattern was found associated with observed humidity pattern throughout the emergence period. The critical mean humidity appeared to be somewhere between 70% and 90%. Most of the heavy showers of rains during the period of emergence were followed by a sharp increase in adult emergence. The effect of rainfall and, therefore, of humidity may clearly be demonstrated with the data for the period between June 26 and 30. During this period, a rise of humidity only by 13% following a heavy shower resulted in a sharp increase in adult emergence from nearly 0 to about 19% (figure 3a). Field emergence data with regard to a total of 1639 adults emerged during the study period (631 in 1982, 584 in 1983 and 424 in 1984) have been pooled to analyze the correlation between relative humidity and adult emergence. Results are presented in figure 3b, in which the curvilinear relationship between these two variables has been shown, along with the plotted scattered diagram. A coefficient of 0·848 strongly suggests a high degree of positive correlation between the relative humidity and adult emergence.

Foregoing observations clearly indicate that a rise in humidity following rainfall acts as an emergence stimulus to the species. However, this is not true for post-monsoon insectary emergence during October and November with almost no rains.

3.3 *Imaginal life*

3.3a *Flight and feeding activity*: Beetles are diurnal and mostly found engaged in

avoid shady areas. The activity, as indicated by the number of feeding, mating and ovipositing adults in the field counted per time-unit of 2 h (table 5) starts in the morning, gradually increases with time and attaining maximum during mid-day, gradually decreases as the sun sets. Activity initially involves walking on the surface of the resting or overnight material, followed by flight. Adults are strong fliers and found actively flying on the foliage, flowers and blossoms of many plants, particularly the larval hosts, usually situated at a height of more than 2 m. These feeding-flights are usually of short duration varying from a few seconds to a few min. They may make circular flights around the branches or trees, often reverse the direction and circle back and forth over and around the inflorescens before landing. Disturbance of any kind during feeding leads the individual to cease the activity of any kind and to fly rapidly upwards.

Adults are flower visitors and do not restrict themselves to any particular part of the flower. However, upon landing on the flower, they tend to proceed towards stamens, which may suggest a preference for stamens or pollen grains. Time spent in feeding on individual flower varies from a few seconds to about 5 min, most probably depending on the size of the flower. Feeding greatly affects survival and unfed adults die much earlier than fed ones under similar conditions, as it was estimated from the laboratory observations of 100 fed and 55 unfed adults (figure 4).

Table 5. Daily average of adults of *N. apicalis* (fractions are converted to nearest whole number) found in different activities in the field during June and July of 1982–1984.

	Average number of adults in different time-units (h)						
Activity	6–8	8–10	10–12	12–14	14–16	16–18	18–20
Feeding	2	9	25	36	23	14	2
Courtship/ mating (in pairs)	0	2	14	29	16	11	0
Ovipositing	0	1	12	25	15	9	0

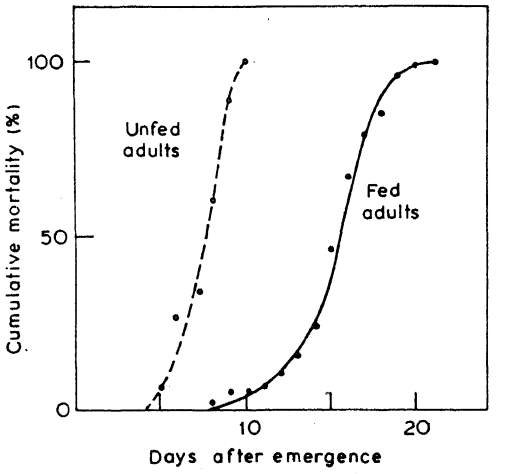

Figure 4. Comparative survival of fed and unfed adults of *N. apicalis* in the laboratory.

3.3b *Sexual behaviour*: Copulation commences soon after emergence, usually on the material from which adults have emerged. Adults of both the sexes are, therefore, sexually mature on emergence as in other Cerambycinae (Saliba 1974; Hosking and Bain 1977; Khan and Maiti 1983; Khan 1984). Later matings take place on trees, foliage, flowers, blossoms or even on the ovipositing material, depending on the opportunity.

In bright sunlight, even up to 40% of the time may be spent in courtship and copulation. Maximum sexual activity in the field, as revealed by the record of highest number of mating pairs, has been observed during mid-day from 1200 to 1400 hours (table 5). Under overcast skies or in diffuse daylight in the laboratory, the rate is correspondingly reduced. The rate is also reduced during afternoon with decreasing sunlight (table 5). Similarly, shady areas are normally avoided by the adults.

In captivity, copulation normally takes place on host-billets provided with adults, on soil and also on the walls of breeding-cages. The duration of actual mating varies from 30 s to 2–3 min, but in most cases, the pair maintains a position of amplexus for a period even up to an hour. Similarly, after copulation and during oviposition, males usually maintain attendance upon females. In the laboratory, not only the same pair is observed to mate several times in a period of 10–15 min, but also both males and females mate with different individuals in succession. All these facts suggest that, as in other Cerambycidae (Linsley 1961; Cannon and Robinson 1981; Khan 1984) multiple-matings occur in this species. The effect of single-mating and multiple-matings on the ecobiology of *N. apicalis* is not known. However, multiple-matings are evident to play an important role in the biology and evolution of Cerambycidae, particularly regarding the potential fecundity (Cannon and Robinson 1981; Khan 1984).

Competition among males for mating is very common and appears to be a factor influencing the length of time an individual male remains in contact with a female. Males, under such disturbances, have mostly been found to respond only by making pushing movements with antennae. No disengagement is observed in copulating pairs, in such cases, the intruder moving away. However, on rare occasions, the mating male may be found to leave the female and chase the intruder. Under such circumstances, usually one or the other male retreats and runs off while the victor engages the female.

3.3c *Oviposition behaviour and fecundity*: After copulation, females are normally observed to disperse in search of suitable ovipositing material. Field observations of marked ovipositing females (table 6) suggest their visit and oviposition on bait logs for a long period. Marked females captured during the later period of these observations are found to lay a considerably lesser number of viable eggs in captivity (table 6). These results clearly indicate that gravid females stay in the immediate vicinity of suitable ovipositing material until all of their fertilized eggs are laid. However, this depends obviously upon the presence of the host-material in abundance in the area, as well as, upon suitable climatic conditions. Similar observations have also been made by Hosking and Bain (1977) in New Zealand and by Khan and Maiti (1982a) in Andaman islands. The time interval between first matings and first oviposition normally varies from 1 to 2 days in newly emerged females.

Females are known to prefer freshly felled host-material for oviposition (Beeson and Bhatia 1939). However, most of the females in the present study have been found

Table 6. Visit and oviposition of a total of 193 marked gravid females of *N. apicalis* on a total of 437 bait host-logs.

	Days of observation after marking and release of the females											
	1	2	3	4	5	6	7	8	9	10	11	12
a (%)	21	29	47	59	63	64	61	53	39	23	12	5
b (%)	9	11	27	35	37	39	31	24	11	6	3	1
c (No.)	89	—	69	—	43	—	29	—	17	—	6	—

a and b, Females visited and oviposited on bait logs respectively; c, number of viable eggs laid per female in captivity after collection from the field (collection was made on alternate days).

Table 7. Field infestation of Sal (*S. robusta*) logs lying (I) in shady areas and (II) in bright sunlight, as well as, (III) of different period lapse after felling by (a) *N. apicalis* and (b) *H. spinicornis*.

	Logs infested by eggs and early larval stages (%)							
			III (Period lapse in weeks)					
	I	II	2	4	6	8	10	12
No. of logs examined	1197	1269	311	392	401	352	277	273
a	3·6	36·5	0·0	29·6	36·4	15·7	3·2	0·0*
b	41·0	7·6	39·0	9·6	0·2	0·0	0·0	0·0*

*The difference between the two is significant at 0·1% level.

to prefer the logs after a month of felling to those felled only a few days ago. Field observations of logs of different period lapse after felling suggested a strong preference of this species for the logs of 4–6 weeks after felling. No infestation was recorded in the logs up to two weeks of felling (table 7). *Hoplocerambyx spinicornis*, which is very common in the area, in contrast, oviposits almost exclusively in freshly felled logs (Beeson and Bhatia 1939). Present observations indicate that *H. spinicornis* prefers logs up to a month after felling, but mostly avoids older logs (table 7). Another worthmentioning behavioural feature of the ovipositing *N. apicalis* females is the avoidance of logs lying in shady areas. The logs lying in bright sunlight appear to be the most suitable materials for *N. apicalis*, which on the other hand are avoided by *H. spinicornis* (table 7).

Oviposition occurs only after the selection of suitable host-material. Prior to oviposition, the female searches suitable oviposition sites by crawling slowly on the host surface with extruded ovipositor. This searching procedure may extend even up to half an hour.

Oviposition extends throughout the life of the adult female, which varies from a few days to about 3 weeks. Observations on the fecundity of this species have not been made in the present study. However, in the laboratory, 6 females have been found to lay 11, 23, 49, 78, 103 and 127 eggs in 1, 2, 4, 5, 8 and 11 days respectively after which they were found dead. The number of eggs laid by a female was found to vary from 5–21 per day.

4. Discussion

Foregoing study clearly reveals that *N. apicalis* does not differ greatly in major biological features, from other shallow sapwood boring Cerambycine beetles not only of Indian subcontinent (Beeson and Bhatia 1939; Khan and Maiti 1983; Khan 1984), but also of the world as a whole (Linsley 1959, 1961), as it is normally expected. Oviposition in batches, larval feeding habit in the subcortical zone, pupation inside the sapwood, excavation of no special exit-tunnel for adult emergence, widely variable developmental period with a prolongation of life-cycle to the second year, pre-monsoon emergence period, flower visiting habit of the adult, sexual maturity on emergence and copulation soon after post-emergence, etc are only a few of the features shared with a considerable number of other Cerambycinae inhabiting the Indian subcontinent.

Similarly, a positive correlation between adult emergence and relative humidity and rainfall is not only evident in *H. spinicornis*, but also in a number of Indian (Beeson and Bhatia 1939) and Maltese (Saliba 1977) Cerambycidae. However, the critical mean humidity triggering the adult emergence of *N. apicalis* still remains tentative and, therefore, demands further investigations for a more specific value. Adult emergence during the period without rains also demands special experimentation for detecting the emergence stimulus in the absence of the effect of humidity and rainfall, although Saliba (1977) and Hosking and Bain (1977) suggested temperature in this regard respectively for the Cerambycinae, *Cerambyx dux* Fald. in Maltese Islands and *Arhopalus ferus* (Muls.) in New Zealand.

The lack of knowledge of the host selection and preference in Cerambycidae restricts us from arriving at any meaningful conclusion of the phenomena in *N. apicalis*. Although further experimentation is mandatory, present observations suggest atleast that *N. apicalis* prefers logs after a month of felling for oviposition, in contrast to *H. spinicornis* which oviposits almost exclusively in the logs within a month after felling. Whatever may be the underlying principle, such an oviposition behaviour must have an adaptive value, atleast, in minimizing the competition with *H. spinicornis* for suitable ovipositing material. Avoidance of logs lying in shady areas may also help in minimization of competition between these two species.

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Micromorphology and cytochemistry of the branchial glands of the freshwater mullets, *Rhinomugil corsula* (Ham.) and *Sicamugil cascasia* (Ham.)

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Abstract. Scanning electron microscopic studies of the gills of *Rhinomugil corsula* and *Sicamugil cascasia* reveal interspecific variations in distribution, density and architectural plan of the mucous glands and the cytochemical nature of the mucus secreted by them. The gill arch and gill filament epithelium of *Rhinomugil corsula* contains numerous mucous cells, but *Sicamugil cascasia* has few mucous gland openings on its gill arch and gill filament epithelium. In both the mullets the secondary lamellar epithelium lacks mucous gland openings, which is discussed.

The presence of blue, blue-red, red and red-blue mucous cells [AB-pH 2·5 periodic acid-Schiff] in the gills of *Rhinomugil corsula* signifies the acid and neutral nature of the glycoprotein of the mucus secreted by them. However, the presence of only blue mucous cells indicates the presence of acid glycoprotein in the mucus of the gills of *Sicamugil cascasia*.

Keywords. Mullets; micromorphology; cytochemistry; mucous glands.

1. Introduction

The gill units are invested with different kinds of epithelia and cells. These cells are designated as branchial glands. The branchial glands perform various functions under normal and experimental conditions.

Several studies have been made on the general organization of gills with special reference to branchial glands (Munshi 1960; Singh and Munshi 1968; Ojha and Munshi 1974). However, little is known on the surface specializations of branchial glands of Indian fish (Hughes and Munshi 1978).

Previous histochemical studies showed that mucous cells from the epidermis and gills of many teleosts contain glycoproteins (Asakawa 1970; Bremer 1972; Zaccone 1972, 1973; Harris *et al* 1973; Carmignani and Zaccone 1974; Ojha and Munshi 1974) but little is known about the different types of glycoproteins present in the mucous cells of fish gills (Fletcher *et al* 1976). Such histochemical analysis is lacking on the gills of Indian species.

The present work is an attempt to demonstrate the surface ultrastructure and the cytochemical nature of the mucus secreted by the branchial glands of the two mullets inhabiting freshwaters of river Ganges.

2. Materials and methods

Live specimens of *Rhinomugil corsula* (30–50 g) and *Sicamugil cascasia* (2–5 g) were collected from the river Ganges and were transported to and maintained in the animal house of the University.

2.1 *Fixation for cytochemical analysis*

The fish were anaesthetized by MS 222 (0·01 gl^{-1}) and the gills were carefully removed, washed in Ringer's and fixed in Bouin's and Zenker's fixatives. After processing, the gill pieces were dehydrated in ethanol, embedded in paraffin and sectioned at 5 μm.

2.2 *Cytochemical tests*

The sections of gills were subjected to various cytochemical tests to demonstrate the chemical nature of the mucus secreted by the branchial glands.

The Schiff's without oxidation technique (Pearse 1968) was used to demonstrate free aldehyde. The periodic acid schiff's (PAS) method (McManus 1946) was employed to demonstrate glycoproteins in branchial glands. A greenish-blue colour reaction with alcian blue (8GS) adjusted to pH 2·5 with 3% acetic acid (Steedman 1950), was employed to identify the nature of acid glycoproteins. Alcian blue at pH 1·0 (Lev and Spicer 1964) was used to differentiate sulphated acid glycoprotein. After staining with alcian blue (pH 1·0), the sections were blotted dry as recommended by Lev and Spicer (1964).

AB-pH 2·5-PAS technique (Jones and Reid 1973a, b) was used to demonstrate the glands secreting different types of glycoproteins.

2.3 *Chemical blockage*

The acetylation technique (Lillie 1954) which blocks the hydroxyl groups forming acetyl esters, and the deacetylation technique (Lillie 1954) which hydrolyses the acetyl esters and unblocks the reactive hydroxyl group were also used.

Methylation (Spicer 1960) which blocks the basophilia of acid glycoproteins giving a negative reaction with alcian blue and subsequent saponification (demethylation) which restores the blue staining of acid glycoproteins with alcian blue, were also employed.

2.4 *Scanning electron microscopic investigations*

For scanning electron microscopy (SEM) 3% phosphate buffered glutaraldehyde was irrigated through the gills of the fish without its prior anaesthetization. This ensured that the pumping action of the gill would quickly rinse the gills. After 5 min the fish seized ventilation and small pieces of gills were fixed in 12% phosphate buffered glutaraldehyde and stored at 4°C for 48 h. The fixed materials were dehydrated and stored in dry acetone. The gill pieces were critically point dried, immediately gold sputted and examined under scanning electron microscope (PSEM/500) at RSIC, Bose Institute, Calcutta.

3. Observations

3.1 *Cytochemistry of branchial glands*

Light microscopy and various cytochemical tests indicate the presence of mucous glands in the gill units of the two mullets.

Table 1. A summary of the histochemical tests performed to show the chemical nature of the specialised branchial glands (mucous glands) of *R. corsula* and *S. cascasia*.

Material	Fixative	Techniques	References	Results	Remarks
Gill	Zenker's fluid	PAS	McManus (1946)	++++R	Mucous glands are PAS positive hence contain carbohydrate
—"—	—"—	PAS acetylation	McManus and Cason (1950)	–	1:2 glycol groups may be present
—"—	—"—	PAS deacetylation	McManus and Cason (1950)	++R	The restoration of the colour indicates the presence of 1:2 glycol groups
—"—	—"—	Schiff without oxidation with periodic acid	Pearse (1968)	–	Free aldehyde is not present
—"—	—"—	Alcian blue pH 2·5	Mowry (1963), Steedman (1950), Warren and Spicer (1961)	+++B	The mucous glands are AB positive and contain acid mucopolysaccharide
—"—	—"—	Alcian blue pH 1·0	Spicer (1960)	+B	Sulphated mucopolysaccharide present in the mucous glands
—"—	—"—	Alcian blue/PAS	Mowry (1956)	++++BR	
—"—	—"—	Mild methylation/ Alcian blue	Spicer (1960)	+B	Acid mucopolysaccharide is present in the mucous glands
—"—	—"—	Mild methylation/ PAS	Spicer (1960)	+++R	It indicates the sulphated nature of AB positive mucous glands
—"—	—"—	Mild methylation/ Alcian blue/PAS	Spicer (1960)	+++BR	
—"—	Zenker's of Bouin's fluid	Alcian blue pH 2·5 PAS	Jones and Reid (1973a, b)	++R; ++RB; ++B; ++BR	Only in *R. corsula* red and red-blue signify the presence of neutral glycoprotein whereas blue or blue-red indicate the presence of acid glycoprotein

B, Blue; BR, blue red; R, red; RB, red blue; +, positive; −, negative.

The PAS positive mucous glands indicate the presence of carbohydrate in mucus, secreted by these specialized branchial glands (table 1). Their negative reaction with PAS, without oxidation with 1% periodic acid, signifies the absence of free aldehyde in the carbohydrate unit of the mucus. They also showed negative results with PAS after acetylation. Deacetylation draws back the PAS positive results indicating the presence of 1:2 glycol-groups. The mucous glands contain glycoproteins and not glycogen, as they resist 1 h treatment with saliva at 37°C and gave PAS positive result after saliva treatment.

The alcian blue tests on the mucous glands at pH 2·5 imparted beautiful colouration, but only a faint reaction was observed at pH 1·0.

The AB-positive mucous glands resist mild methylation for 12 h at 37°C which was clarified by AB, AB-PAS and PAS techniques. These reactions indicate the sulphated nature of the AB-positive mucous glands. Methylation gave a negative reaction with AB-pH 2·5 and subsequent saponification restored the blue staining of acid glycoproteins with AB-pH 2·5.

3.2 *Assessment of staining and types of glycoproteins*

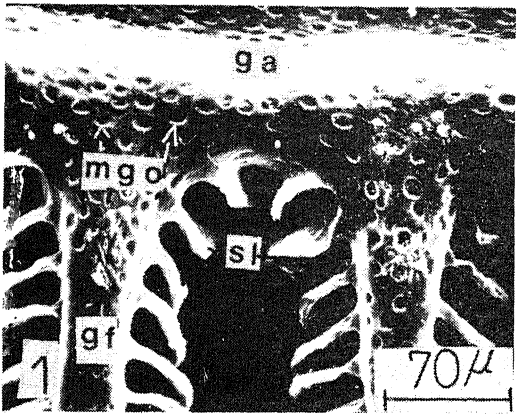
In *R. corsula* 4 types of mucous glands (blue, blue-red, red and red-blue) were identified by AB-pH 2·5-PAS technique (table 1). However, in *S. cascasia* colour differentiation could not be observed. Blue or blue-red mucous glands signify the presence of acid glycoproteins and red and red-blue ones indicate the presence of neutral glycoproteins in mucus secreted by these glands.

3.3 *SEM studies*

The epithelia covering the gill head and the gill filaments of the two mullets are differentiated into glandular and non-glandular parts. In *R. corsula* large number of mucous glands surrounded by microridged epithelial cells are discernible on the gill-head and gill filaments (figures 1, 2). The laminated microridges penetrate into the mucous glands (figures 3, 4). The orientation of the mucous glands and the pattern of microridges in the primary epithelial cells are similar to those of the gill arch. The tip of the gill filaments also shows the same distribution pattern and architectural plan of the mucous glands and microridged epithelial cells (figure 5).

Figures 1–8. **1.** A part of the gills of *R. corsula* showing many mucous gland openings on the gill arch and gill filament epithelia. Secondary lamellae are also seen. **2.** Base of the gill filament of *R. corsula* showing mucous gland openings on its epithelium. Secondary lamellae are also seen. **3 and 4.** A part of the gill filament epithelium of *R. corsula* showing mucous gland openings and microridged epithelial cells. Microvilli are also seen in the centre of some microridged epithelial cells (figure 4). **5.** The tip of a gill filament of *R. corsula* showing mucous gland openings. Secondary lamellae are also seen. **6.** Base of the gill filament of *S. cascasia* showing mucous gland openings and microridged epithelial cells. **7 and 8.** A part of the gill filament epithelium of *S. cascasia*. **7.** Mucous gland openings and microridged epithelial cells. **8.** Enlarged view of the mucous gland opening.

(mgo, Mucous gland opening; ga, gill arch; gf, gill filament; sl, secondary lamellae; mrc, microridged epithelial cells; mv, microvilli; tgf, tip of a gill filament).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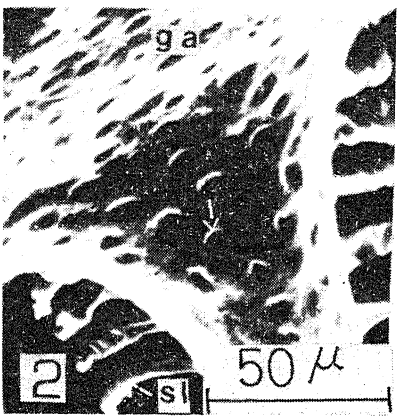
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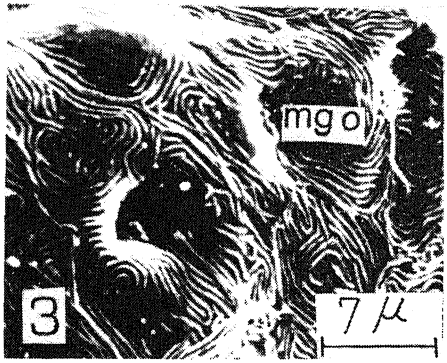
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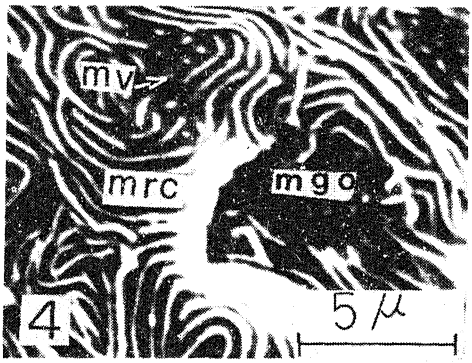
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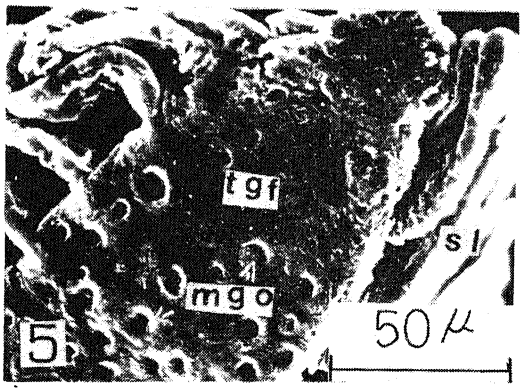
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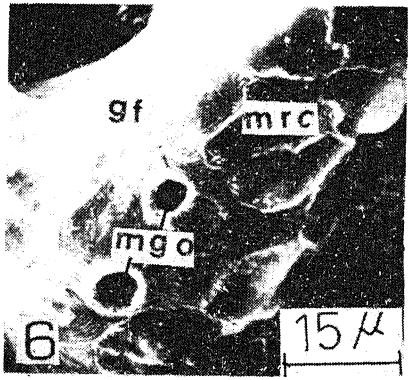
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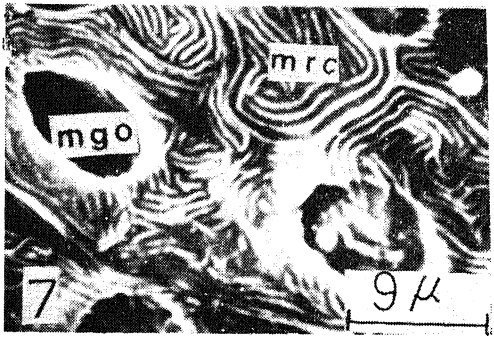
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In *S. cascasia*, the gill arch and the gill filament are provided with mucous glands and microridged epithelial cells (figure 6) but the number of mucous glands are quite low compared to that of *R. corsula*. Each mucous gland has a well-defined rim-like opening (figures 7, 8).

4. Discussion

SEM and cytochemical studies reveal interspecific variations in various components of the gills of the two mullets. *R. corsula* has more number of mucous glands in the gill arch than found in *S. cascasia*. The architectural plan of the microridged epithelial cells of *R. corsula* is also different from those of *S. cascasia*. The gill arch and its histology thus appear to be characteristic for these two fish species.

The SEM picture of the gill structure indicates differences in architecture. The differences are mainly in the density and distribution of the mucous glands and the architectural plan of microridges in the primary epithelial cells. The architectural plan of the primary epithelium was similar to that investing the gill arch. From these observations it appears that fishes have inter- and intra-specific variations in density, distribution and architectural plan of the mucous glands and microridged primary epithelial cells.

Each secondary lamella is lined externally by secondary epithelium, which is quite different from the primary epithelium. The secondary epithelium lacks mucous glands and microridged cells which is an adaptation for efficient gaseous exchange. Microridged cells filled with mucus would normally increase the water-blood pathway in the gills which is not favourable for efficient gaseous exchange. Smooth epithelial cells in the secondary epithelium decreases the water-blood pathway and therefore increases gas-exchange efficiency of the fish gills.

4.1 *Histochemical analysis of branchial glands*

In the gill epithelium of *R. corsula*, blue, blue-red, red-blue and red mucous cells were demonstrated by AB-pH 2·5/PAS technique. The range of glycoproteins by this method was similar to that described at epithelial tissue sites in mammals (McCarthy and Reid 1964; Spicer *et al* 1974). Blue and blue-red mucous cells in the gills of *R. corsula* suggest the presence of predominantly acid glycoprotein and those staining red-blue and red predominantly neutral glycoproteins. The number of mucous glands secreting acid glycoprotein was more than those secreting neutral glycoproteins. Similar results have been obtained by Fletcher *et al* (1976) in the gills of plaice, flounder and rainbow trout.

The predominant type of acid glycoprotein in the mucous cells of branchial epithelium has been found to vary between species (Porcelli and Novelli 1970; Zaccone 1972, 1973) consistently. In the gills of *S. cascasia* only blue mucous glands were detected by AB-pH 2·5/PAS technique. This indicates that the mucous cells of this species secrete only acid glycoprotein. The glycoproteins produced by branchial mucous cells thus show interspecific variations.

Mucus secretion in fish is usually assumed to be protective (Jakowska 1963). The secretion further helps to reduce friction (Rosen and Cornford 1971) and to check bacterial and fungal growth. According to Hughes and Wright (1970) the mucus film

covering the secondary gill lamellae in teleost as an important function in relation to gas, ionic and water exchange at the gill surface. The sulphated acidic mucus produced by mucous glands of *R. corsula* and *S. cascasia* helps to check bacterial and fungal growth on gills. The mucus also protects the gills from mud and fine sediments and thus, gills work efficiently in maintaining gaseous and ionic regulation. In *R. corsula* and *S. cascasia* no mucus was found to be present in the secondary epithelium of the lamellae. Similar finding has been reported by Fletcher *et al* (1976) in the gills of *Salmo gairdneri.*

SEM and histochemical studies point out the inter- and intra-specific variations in the density, distribution and architectural configuration of the branchial glands of the two species of mullets studied presently.

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Nature of resistance in selected rice varieties and population fluctuation of green leafhoppers, *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stål)

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Abstract. In multiple choice tests with 30-day-old plants of resistant and susceptible varieties, both the species of green leafhoppers, *Nephotettix virescens* (Distant) and *Nephotettix nigropictus* (Stål) exhibited relative non-preference to highly resistant varieties (Ptb 2, Ptb 7 and Ptb 18) both for settling and oviposition. Even when 100 first instar nymphs were caged on individual 25-day-old plants of highly resistant varieties, the plants suffered very low damage and also induced high mortality of nymphs. Different ages of the plants had no influence on the antibiosis mechanism of resistant varieties. Feeding behaviour studies revealed that both the species made more punctures and excreted less honeydew while feeding on resistant varieties than on susceptible ones. Histological studies indicated no mechanical barrier for feeding in resistant varieties.

Field investigations indicated that resistant varieties viz Ptb 18, Ptb 2 and Ptb 7 harboured less population of green leafhoppers and had relatively nil or very low incidence of tungro virus disease. An year round survey in the rice ecosystem and marshy habitat where *Leersia hexandra* grows in abundance revealed that although both the species coexisted in rice fields, *Nephotettix nigropictus* alone was present in the marshy habitat.

Keywords. *Nephotettix virescens*; *Nephotettix nigropictus*; nature of resistance; population fluctuation.

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1. Introduction

The current trend in agricultural pest control programmes in various rice growing countries is towards evolving a sound pest management system. It requires a proper understanding and implementation of the population regulating factors such as cultural control including growing resistant varieties. In India, screening for resistance to green leafhoppers, *Nephotettix virescens* (Distant) and *N. nigropictus* (Stål), was initiated at the national headquarters of the All India Coordinated Rice Improvement Project (AICRIP 1969) and also at the Central Rice Research Institute (CRRI 1970).

In view of the increased importance of green leafhoppers in various states of the country, we investigated the insect-host interaction at the Directorate of Rice Research, Hyderabad (formerly AICRIP). Earlier, Viswanathan and Kalode (1984) reported 10 rice varieties to be greatly detrimental to insect bionomics using 1st instar nymphs and adults of both green leafhoppers (*N. virescens* and *N. nigropictus*). We now describe the mechanism of resistance in selected rice varieties and population fluctuation in rice ecosystem.

2. Materials and methods

2.1 *Preference/non-preference mechanism*

2.1a *Settling and ovipositional response to susceptible TN 1 rice plants of different ages*: TN 1 plants of different ages were grown randomly in a wooden tray. Plants

of the same ages were replicated 5 times. Gravid females were released in a cage containing the tray. Observations on settling preference and egg laying were made by counting the insects and dissecting plants for recording number of eggs.

2.1b *Preference/non-preference response of adults to selected rice varieties*: Eight plants of each variety (Ptb 18, Ptb 2, Ptb 7 and TN 1) were grown randomly in 3 replications in wooden trays at a spacing of 10×10 cm and only the mother tiller was allowed to grow. When plants were 30-day-old, a total of 320 insects in a sex ratio of 1 : 1 were released and observations were taken on the insect settling pattern at every hour upto 7 h and at 24, 30, 48 and 54 h.

2.2 *Studies on antibiosis*

2.2a *Effect of higher population on antibiosis reaction*: The high antibiosis reaction exhibited in the selected resistant varieties (Ptb 18, Ptb 2 and Ptb 7) was further confirmed by caging 100 first instar nymphs per 25-day-old plants. Seven replications were maintained for each test variety. Damage caused was assessed at 5, 10, 15 and 20 days after insect release and the damage percentage was calculated as follows

$$\text{Damage } (\%) = \frac{\text{No. of leaves damaged}}{\text{Total No. of leaves}} \times 100$$

2.2b *Effect of plant age on antibiosis*: To study the influence of plant age on antibiosis, known number of first instar nymphs were caged on 10, 25, 45 and 90-day-old plants. Three replications were maintained for each age group of varieties. Surviving nymphs were counted daily until 7 days.

2.3 *Population buildup on selected varieties*

2.3a *Greenhouse test*: The population buildup was studied by releasing 5-day-old 5 females and males on 25-day-old plants of test varieties. Each variety was replicated 10 times, population buildup was recorded 35 days after infestation.

2.3b *Field test*: To determine population fluctuation of the two green leafhopper species and the incidence of rice tungro virus, a field trial was conducted at the AICRIP experimental farm during *kharif* season. In addition to the 3 highly resistant varieties, one moderately resistant variety (IR 8) and one susceptible variety (TN 1) were also included. The selected varieties were planted in a randomized block design and the plot size was 16 sqm. Each variety was replicated thrice. Bordering the test varieties, the susceptible TN 1 was planted horizontally so as to increase the insect population. Ten days after transplanting, 50 oviposited pots from greenhouse were kept on both sides of the plots along TN 1 lines to increase the insect pressure in the experimental field. Observations were made on 30 hills of each variety at every 10 days upto 80 days after transplanting. Tungro virus incidence was recorded at 60 days after transplanting based on disease symptomology.

2.3c *Population fluctuation*: Using a sweepnet, the two species of green leafhoppers were sampled periodically in rice fields, rice fallows and surrounding

swampy areas to obtain supplementary information on natural population fluctuations.

2.4 *Feeding behaviour on selected varieties*

2.4a *Honeydew excretion*: In greenhouse experiments, both third instar nymphs and adults were kept without food for 2 h and caged separately on single leaf in special glass cages. Each variety was replicated 5 times. Two male and female insects each were caged when adults were used for test. Similar tests were carried out by caging 4 third instar nymphs. Honeydew droplets were collected on Whatman filter paper by adopting technique similar to that described by Reddy and Kalode (1985). After 24 h, the insects were removed and the extent of honeydew excreted was visually assessed by spraying the Whatman filter paper with ninhydrin reagent (0·02%), which reacts as pinkish yellow spots with the amino acids present (IRRI 1968).

2.4b *Probing marks*: The number of feeding marks made on the test variety was determined by caging a single insect for 6 h by restricting it on a single leaf of 25-day–old plant. There were 5 replications. Then the leaf was dipped in 1% erythrosin solution which stained the feeding marks as per the method suggested by Naito (1964).

3. Results and discussion

3.1 *Preference/non-preference mechanism*

Greenhouse investigations in relation to plants of different ages of the susceptible variety TN 1 showed that *N. virescens* preferred 30-day–old plant, while *N. nigropictus* preferred 40-day–old plant both for settling and egg laying (figure 1). Multiple choice studies using resistant and susceptible varieties indicated that highly resistant varieties were relatively less preferred by both the species for settling as well as egg laying. The insects did alight on the resistant varieties (figure 2), but only by about 7 h after their release, majority of them were found to locate the suitable host. Thus, selection of a suitable host by these species appeared to be a passive one. In similar studies Cheng and Pathak (1972) noted that the green leafhopper (*N. virescens*) differed in their preference for various varieties. In spite of adult-non-preference for different varieties, several of the resistant varieties received as many eggs as the susceptible variety. Likewise Choi *et al* (1979) reported for brown planthopper that resistant varieties which were non-preferred for feeding did not exhibit the same trend towards oviposition. The preference response of the insect to a variety is more pronounced after 48 h of the infestation (IRRI 1977). Such response is presumed to be caused by the lack of certain stimuli or by the presence of a strong repellent in resistant varieties.

3.2 *Studies on antibiosis mechanism*

3.2a *Effect of higher insect population on antibiosis*: The detailed studies with selected resistant varieties (Ptb 2, Ptb 7 and Ptb 18) revealed a high degree of anti-

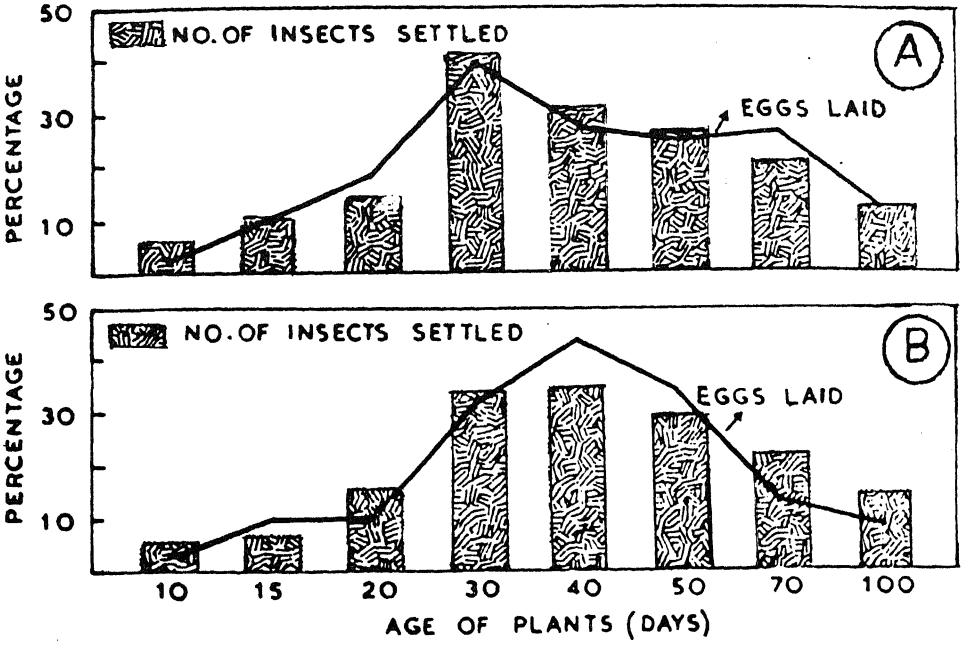

Figure 1. Preferential response for settling and oviposition by *N. virescens* (**A**) and *N. nigropictus* (**B**) for plants of different ages of the susceptible rice variety TN 1.

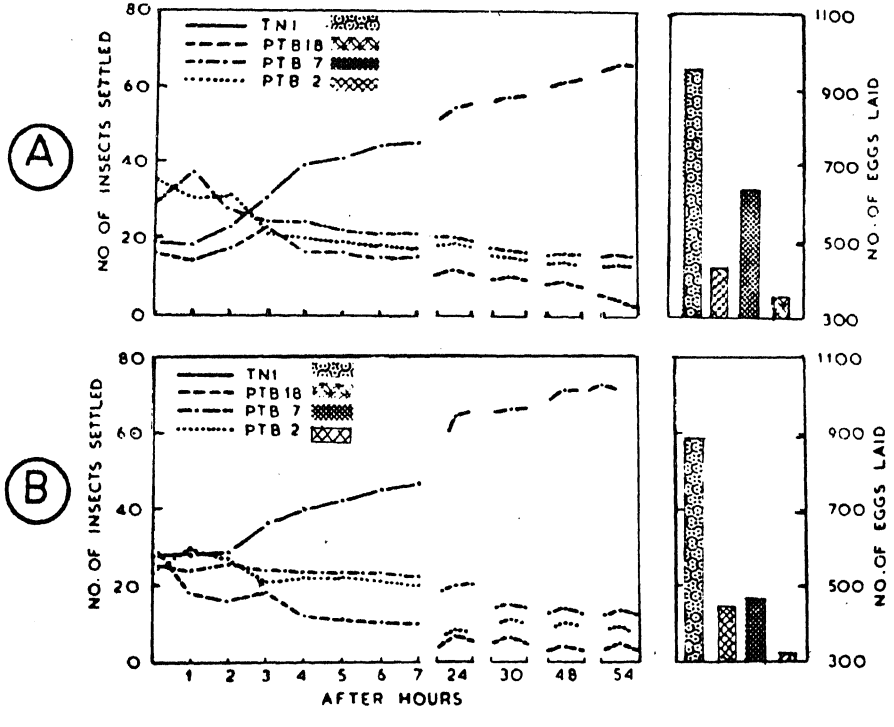

Figure 2. Preferential response for settling and oviposition by *N. virescens* (**A**) and *N. nigropictus* (**B**) for different resistant and susceptible rice varieties.

biosis to both *N. virescens* and *N. nigropictus* (Viswanathan and Kalode 1984). Even with release of 100 first instar nymphs per plant, in the present studies, the damage on resistant varieties was considerably low (figure 3) since there was a high mortality of insects and only meagre population could survive on these varieties. Among the highly resistant varieties, Ptb 18 and Ptb 2 proved to be relatively better than Ptb 7 for both the species. It was evident that resistant varieties could drastically reduce the insect population caged on them. This population reduction could primarily be attributed due to their high antibiosis. Such type of population reduction in *N. virescens* and *Nilaparvata lugens* (Stål) due to antibiosis mechanism is known in rice varieties (Cheng and Pathak 1972; Sogawa 1973; Karim 1975; Karim and Pathak 1978; Heinrichs and Rapusas 1984). High rate of initial nymphal mortality on resistant varieties has been noted for *N. cincticeps* (Kawabe 1985). The present results also reveal this trend for both *N. virescens* and *N. nigropictus*. However, high level of initial nymphal mortality was not observed in case of brown planthopper as only

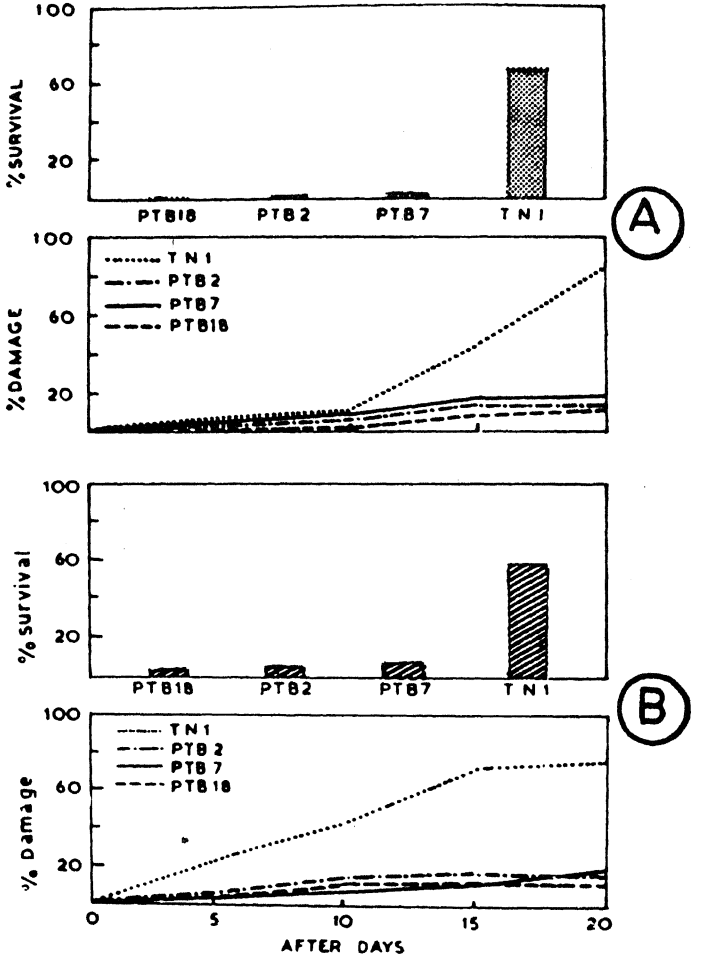

Figure 3. Plant damage (%) and survival of *N. virescens* (**A**) and *N. nigropictus* (**B**) on different rice varieties when caged with 100 first instar nymphs.

19·5–30% nymphal mortality was recorded upto 6th day of caging on resistant varieties (Reddy and Kalode 1985).

3.2b *Effect of plant age on antibiosis*: Age of the plant showed no significant influence on antibiosis mechanism in highly resistant varieties studied. On the susceptible TN 1, higher nymphal population (88–96·5%) of both the species survived for 7 days after release on plants of different age groups. But on resistant Ptb 18, within 5 to 6 days, there were no surviving insects (*N. virescens*) on 10, 25, 45 and 90 day old plants. Similarly, plants of different ages of other resistant varieties (Ptb 2 and Ptb 7) did not show any significant differences in relation to survival of nymphs. No surviving *N. nigropictus* nymphs were observed beyond 4 days on Ptb 18 plants of 90 day old while on other ages of the same variety, a low population was recorded. At the end of 7 days, either there were no surviving insects or meagre population on plants of different ages of Ptb 2 and Ptb 7. The observations made at the International Rice Research Institute (IRRI 1968) support present findings where the age of the varieties did not alter the resistance of rice varieties to green leafhoppers and brown planthoppers. In contrast, some amount of fluctuation in levels of antibiosis with the growing stages of the resistant rice varieties has been reported for *N. cincticeps* (Kishino and Ando 1979). Cartier (1963) noted that absolute resistance of peas to *Acyrthosiphon pisum* (Harr.) increased with age of the plants, but relative varietal differences remained constant during most of the life of the host plant.

3.3 *Population buildup on resistant and susceptible varieties*

3.3a *Greenhouse test*: The population increase from 5 pairs at 35 days of release was 297 (115 adults) in case of *N. virescens* and 123 (69 adults) in case of *N. nigropictus* on susceptible TN 1 as compared to 7 (*N. virescens*) and 11·5 (*N. nigropictus*) per plant of Ptb 7. On other resistant varieties both the species did not survive except 1·5 insects (*N. nigropictus*)/plant of Ptb 2.

3.3b *Field test*: High level of resistance in Ptb 18, Ptb 2 and Ptb 7 as observed in the greenhouse was further confirmed in field experimentation (figure 4). Both the species indicated close similarity in population buildup showing peaks at 60 days after transplantation though the number of *N. nigropictus* was relatively less than that of *N. virescens* particularly on susceptible variety TN 1 and moderately resistant IR 8. Thus highly resistant varieties could suppress the field population of the green leafhoppers. Similar observations were made by Jayaraj (1967a) who noted that *Empoasca flavescens* (F) populations were more on susceptible and tolerant castor varieties than on resistant varieties. Root and Olson (1969) also found that host species and varieties had a definite influence on rate of population development of the aphid, *Bravicoryne brassicae* (Lin.).

It is interesting to note that no tungro virus disease symptoms were manifested on varieties Ptb 18 and Ptb 2, while it was considerably low (4·4%) on Ptb 7 (table 1). Probably, the above varieties did not suffer by this disease because of their high resistance and consequent less insect population. This again upholds the view that the use of varieties highly resistant to green leafhoppers could possibly check tungro spread.

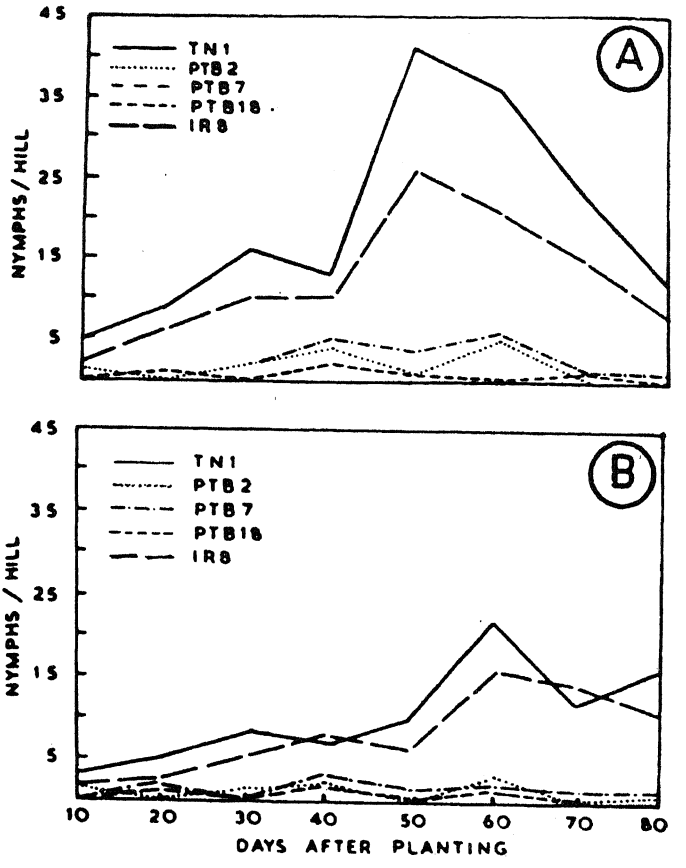

Figure 4. Population fluctuation of *N. virescens* (**A**) and *N. nigropictus* (**B**) on different rice varieties in the field experiment.

Table 1. Percentage of plants infected with tungro disease in field studies with selected rice varieties.

Variety	Damage rating	Plants infected with tungro (%)*
Ptb 2	Resistant	0
Ptb 7	"	4
Ptb 18	"	0
TN 1	Susceptible	97
IR 8	Moderately resistant	90

*Average of 3 replications.

3.3c *Pattern of population fluctuation in rice fields in marshy habitat:* Observations made in rice fields (figure 5) revealed that during August to September, both the species were comparatively meagre but started building up from September onwards. *N. virescens* exhibited 3 peaks from November to January. Similar increase of *N. nigropictus* was observed, except during November. However, the population of

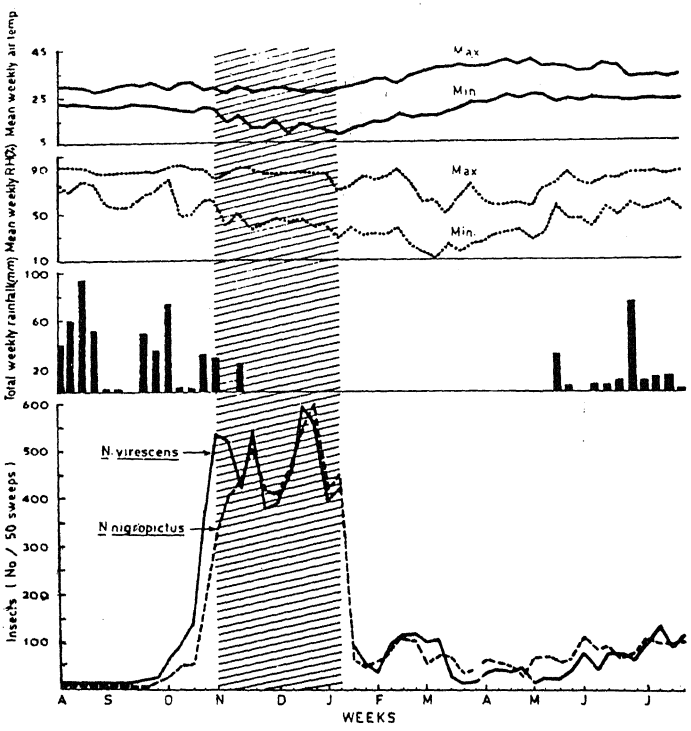

Figure 5. Population fluctuation of *N. virescens* and *N. nigropictus* adults in rice fields.

both the species declined from January 2nd week onwards upto 4th week of July recording low population. During peak periods the maximum temperature and relative humidity were 29–39°C and 80–90%, respectively.

Survey of the marshy habitat showed that *N. virescens* was completely absent throughout. On the other hand, *N. nigropictus* was found building up from October onwards with a peak in March which was in far greater number in comparison to rice fields. However, there was a fall in population from second week of April in this habitat. The main host plant species in this situation was *Leersia hexandra*. *L. hexandra* was found to be the most suitable host for *N. nigropictus* (Viswanathan and Kalode 1986).

3.4 *Feeding behaviour*

3.4a *Amount of feeding—honeydew excretion*: Studies on the honeydew excretion of the two species feeding on resistant and susceptible varieties revealed that both the species fed less and excreted less on highly resistant varieties (table 2). Comparable observations have been made with resistant varieties by Cheng and Pathak (1972) with green leafhopper and in case of brown planthopper by Sogawa (1973), Kalode and Krishna (1979) and Reddy and Kalode (1985). In contrast, the honeydew excreted by *N. cincticeps* on resistant rice plants was by far more in quantity than those on susceptible plants (Kawabe 1985). Recent studies at IRRI have shown that though

Table 2. Relative amount of honeydew excreted on selected rice varieties by green leafhoppers.

	Relative amount of honeydew excreted			
	N. virescens		*N. nigropictus*	
Variety	Nymph	Adult	Nymph	Adult
Ptb 2 (R)	+	+	+ +	+
Ptb 7 (R)	+ +	+	+	+
Ptb 18 (R)	+ +	+	+	−
TN 1 (S)	+ + +	+ + +	+ + +	+ + +

+ + +, Large amount; + +, moderate amount; +, trace amount; −, nil; R, resistant; S, susceptible.

Table 3. Feeding punctures made by *N. virescens* and *N. nigropictus* adults on the leaf blades of selected varieties.

	Punctures/insect/h					
	N. virescens			*N. nigropictus*		
Variety	Min	Max	Mean	Min	Max	Mean
Ptb 18 (R)	6·1	12·5	9·2	5·8	13·2	10·1
Ptb 2 (R)	5·3	10·2	7·1	6·1	14·7	9·3
Ptb 7 (R)	4·8	9·6	6·6	5·2	10·4	7·1
TN 1 (S)	3·2	4·7	3·4	4·4	5·1	4·6

R, Resistant; S, susceptible.

the total area of honeydew spots were similar when green leafhopper fed on resistant or susceptible varieties, xylem feeding increased with the level of resistance (IRRI 1982).

3.4b *Amount of feeding—probing marks*: Both *N. virescens* and *N. nigropictus* were found to make more feeding punctures (6·6–10·1) on resistant varieties as compared to 3·4–4·6 on susceptible varieties (table 3). Similar observations have also been recorded in rice varieties to brown planthopper and green leafhopper (Sogawa and Pathak 1970; Cheng and Pathak 1972; Karim 1975; Reddy and Kalode 1985; Heinrichs *et al* 1985; Khan and Saxena 1985b). Histology of fed tissue showed that there were no physical or mechanical barriers to prevent the insect feeding on resistant varieties where majority of the stylet sheaths reached vascular bundles (table 4). Sogawa and Pathak (1970) also did not find any mechanical barrier to feeding by *N. lugens* in the resistant variety, Mudgo. Cheng and Pathak (1972) concluded that resistance to *N. virescens* cannot be attributed to mechanical factors. Similar conclusions were drawn against *N. cincticeps* by Oya (1980) and Oya and Saito (1981). However, Peraiah *et al* (1979) stated that brown planthopper resistant varieties ARC 6650 and Ptb 33 had thicker hypodermis and an extra sclerenchymatous band in the stems which offered mechanical resistance to the insect. Resistance to *E. flavescens* in castor varieties was attributed to the anomalous thickening around vascular bundles by Jayaraj (1967b).

Table 4. Nature of salivary sheaths produced by *N. virescens* and *N. nigropictus* on selected rice varieties.

	Rice varieties							
	N. virescens				*N. nigropictus*			
Stylet sheath	TN 1 (S)	Ptb 2 (R)	Ptb 7 (R)	Ptb 18 (R)	TN 1 (S)	Ptb 2 (R)	Ptb 7 (R)	Ptb 18 (R)
Total No. of salivary sheath observed	98	107	115	90	78	91	120	67
Percentage salivary sheaths with 2 branches	2	0	4	6	1	0	2	1
Termination of salivary sheath (%)								
Phloem	68	56	71	71	80	66	79	74
Xylem	12	25	22	13	12	10	15	14
Parenchyma	20	19	7	16	8	24	6	12

S, Susceptible; R, resistant.

Thus, the above observations made on the feeding behaviour of *N. virescens* and *N. nigropictus* suggest that the resistance should primarily be due to certain biochemical factors which adversely affect the normal feeding sequence on resistant varieties. This is supported by Khan and Saxena (1985b) who showed that *N. virescens* fed primarily from the phloem of susceptible variety TN 1 but switched to the xylem on resistant varieties. In addition, the insects feeding behaviour on TN 1 plants could be disrupted by spraying the plant by steam distillate extract of resistant ASD 7 (Khan and Saxena 1985a). Further, it is evident that resistant varieties can be effectively used to suppress pest buildup in pest management programmes.

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FOREWORD

The multidimensional concept of species has come to occupy a predominant place in the study of life sciences and the increasing problems confronted in biocontrol programmes, as well as the impact of biotypes or siblings in insect vector species, not to mention the frequency of occurrence of intraspecific diversity in several pest species, have led to the involvement of ecobehavioural, physiological, cytogenetic and biochemical approaches towards a better assessment of the concerned species. The increasing consciousness regarding the need for such an integrated approach to biosystematic studies has never been more acute than it is today. Biosystematics has emerged as an interdisciplinary area of research in life sciences, with considerable impact on applied biology, in particular in the fields of agriculture, forestry, medical and veterinary fields. Such an orientation appears essential in view of the species concept occupying a central place in contemporary biological thought. Being the most important level of integration in the organic world, consistent efforts have been and are being made to redefine and reassess the true nature of the species which are today recognized as distinct ecological, behavioural and genetic units. Besides accurate species determination, a knowledge of population biology of species will enable a better understanding not only of the generic and species complexes, but also of the intraspecific categories.

An assessment of the consequences of individual variation for differential reproductive success in natural population of insects has become obligatory for a better understanding of species. The recognition of the dynamic, plurimodal configuration of species, as against the static, unimodal concept has often been emphasized for a better appreciation of the dimensions of species. Phenotypes resulting through successive interactions of the individuals of a genotype with the environment, involve the entire range, the whole repertoire of various pathways of development that may occur in the carrier of a given genotype in all environments — favourable or unfavourable, natural or artificial. This is not known for most species. Intraspecific diversity enables the combination of resources scattered in time and space to provide a composite niche for a species. The importance of systematic entomology therefore solidly rests on the necessity to understand how ecosystems function and in particular situations what organisms are involved in specific biological processes. For this understanding the species has to be properly identified. It is needless to emphasise that there is an increasing tendency for the occurrence of biotypes in species reacting differently under different situations and this has posed serious problems in applied entomology.

Another aspect of polymorphism relates to the niche width variation hypothesis which interprets that higher frequency of polymorphism is in the central part of the range of a species in view of the greater necessity of niches occupied by central populations, so that greater number of inversion types occur in this area — an aspect deserving close scrutiny. The correlation of electrophoretically detectable enzyme polymorphism and morphological variation also contributes towards a better understanding of the dimensions of species.

The above mentioned dimensions of species are illustrated with examples from diverse groups of insects, presented at the Workshop on Biosystematics of Insects, held at the Entomology Research Institute, Loyola College, Madras during 27-30 April 1987 and sponsored by the Department of Science and Technology, New Delhi.

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Multifaceted approach to evaluate the relationship among closely related forms of *Drosophila*

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Abstract. *Drosophila* is a suitable system to study different facets of population differentiation. *Drosophila sulfurigaster*, *Drosophila bilimbata*, *Drosophila albostrigata*, *Drosophila neonasuta* and *Drosophila pulaua* are morphologically indistinguishable members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila*. They are distributed in different parts of south east Asia. The evolutionary inter-relationship between these closely related forms will be discussed with reference to karyotypes, heterochromatin, satellite DNA, population fitness, ecogenetic divergence and isozyme variations.

Keywords. *Drosophila*; karyotypes; satellite DNA; fitness; resource utilization; isozymes.

1. Introduction

Drosophila is no longer the queen of genetics, as at one time it was. It remains, however, probably the best material for studies on evolutionary and population genetics (Dobzhansky 1970). *Drosophila* has emerged out as a paramount fast breeding diploid bisexual organism for the experimentalist to dissect out and study various facts of evolution. Recently, Templeton (1981) has critically discussed the population genetic mechanisms of speciation. He has cited relevant references to demonstrate that speciation can occur in the absence of, or is uncorrelated in some groups with karyotypic change, DNA sequence divergence, enzyme differentiation, morphological change and shifts in niche or habitat. It has been realized that there is no universal joint pattern relative to speciation. In the words of Dobzhansky (1970) 'the evolution of every phyletic line yields a novelty that never existed before and is unique, unrepeatable and irreversible proceeding'. Therefore one cannot imagine an universal marker to define the process of speciation and it has to be treated as a multidimensional process. In view of this, a multifaceted approach has been made to understand the nature of differentiation among closely related forms of *Drosophila*. The members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila* form the material for the present investigation.

2. Materials and methods

Nasuta subgroup of the *immigrans* species group is an assemblage of morphologically almost identical species and sub-species of *Drosophila*. Taking into cognizance the pioneering work of Wilson *et al* (1969) and their observations, Nirmala and Krishnamurthy (1973) have divided the *nasuta* subgroup into 3 exophenotypic complexes. They are (i) frontal sheen complex which includes the members with silvery sheen over the entire frons; (ii) orbital sheen complex which includes the species with silvery markings confined to the sides of the frontal orbits and (iii) species without such markings.

The present paper deals with the members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila*. The extensive cytological and hybridization investigations of Wilson *et al* (1969), Nirmala and Krishnamurthy (1973) and Ranganath and Krishnamurthy (1976) have resulted in the recognition of the following members of the orbital sheen complex:

(i) *D.s. sulfurigaster* Wilson *et al* 1969 (*D. sulfurigaster* Duda 1923);
(ii) *D.s. bilimbata* Wilson *et al* 1969 (*D. bilimbata* Bezzi 1929);
(iii) *D.s. albostrigata* Wheeler 1969;
(iv) *D.s. neonasuta* Ranganath and Krishnamurthy 1976 (*D. neonasuta* Nirmala and Krishnamurthy 1973);
(v) *D. pulaua* Wheeler 1969;
(vi) *D. nixifrons* Tan, Hsu, Sheng, 1949.

The 4 sub-species of *D. sulfurigaster* are allopatric. *D.s. sulfurigaster* is a dominant member in Papua, New Guinea. *D.s. bilimbata* is widely scattered in many islands of Pacific ocean. *D.s. albostrigata* is a common member in different parts of south east Asia like Ceylon, Thailand, Burma and Philippines. *D.s. neonasuta* is found in some parts of Peninsular India. *D. pulaua* is a common species in Malaysia (Wilson *et al* 1969; Nirmala and Krishnamurthy 1973; Ranganath and Krishnamurthy 1976; Siddaveere Gowda *et al* 1977; Kitagawa *et al* 1982). *D. nixifrons* was reported from China and it was not available for detailed investigations.

The flies of 4 sub-species of *D. sulfurigaster* and *D. pulaua* are morphologically indistinguishable. In this communication an attempt has been made to report and discuss the extent of similarities and differences among these siblings of the orbital sheen complex of *Drosophila* involving different facets of their organization.

3. Results and discussion

3.1 *Karyotypic organization*

Karyotype is a useful marker to evaluate the evolutionary relationships between species or groups of species. Variations in metaphase chromosomes are often striking in the genus *Drosophila*. A number of studies have demonstrated that this endophenotype is more variable than other morphological characters in *Drosophila* (Pimpinelli *et al* 1976; Gatti *et al* 1976; Lemeunier *et al* 1978; Baimai 1980; Baimai and Chumchong 1980; Lakhotia and Mishra 1980; Ranganath and Hagele 1982; Singh and Gupta 1982; Baimai *et al* 1983; Gupta and Kumar 1986; Mahan and Beck 1986). Recently, Ushakumari and Ranganath (1986a) have reported the karyotypes of the 5 members of the orbital sheen complex of *Drosophila*, viz *D.s. sulfurigaster*, *D.s. bilimbata*, *D.s. albostrigata*, *D.s. neonasuta* and *D. pulaua*. The diploid number in these forms is $2n = 8$. In general, the metaphase chromosome complement of a female consists of one pair of metacentrics (chromosome 2), two pairs of acrocentrics (chromosomes X and 3) and one pair of dots (chromosome 4). In males, one of the acrocentric X chromosome is replaced by a submetacentric chromosome Y.

Chromosome banding is a useful technique to localize heterochromatin and to study the evolutionary changes, if any, in the architecture of the chromosomes. In the genus *Drosophila*, it is stated that heterochromatin is more divergent than external

morphological characteristics (Holmquist 1975). In the recent years, C-banding has been frequently used as an approach to study variations in amount and distribution of heterochromatin in the karyotypes of related species and has yielded information useful in resolving karyotypic differences (Kaul *et al* 1978). Diagrammatic summary of metaphase chromosomes of the members of the orbital sheen complex of *Drosophila* after C-banding is shown in figure 1. Table 1 provides the data on the quantum of C-band regions present in different chromosomes and in different strains under study. Chromosomewise comparison between these *Drosophila* forms show significant differences. The acrocentric X chromosome of all these forms carry a block of heterochromatin at the centric region, but the quantum of it ranges from about 48%

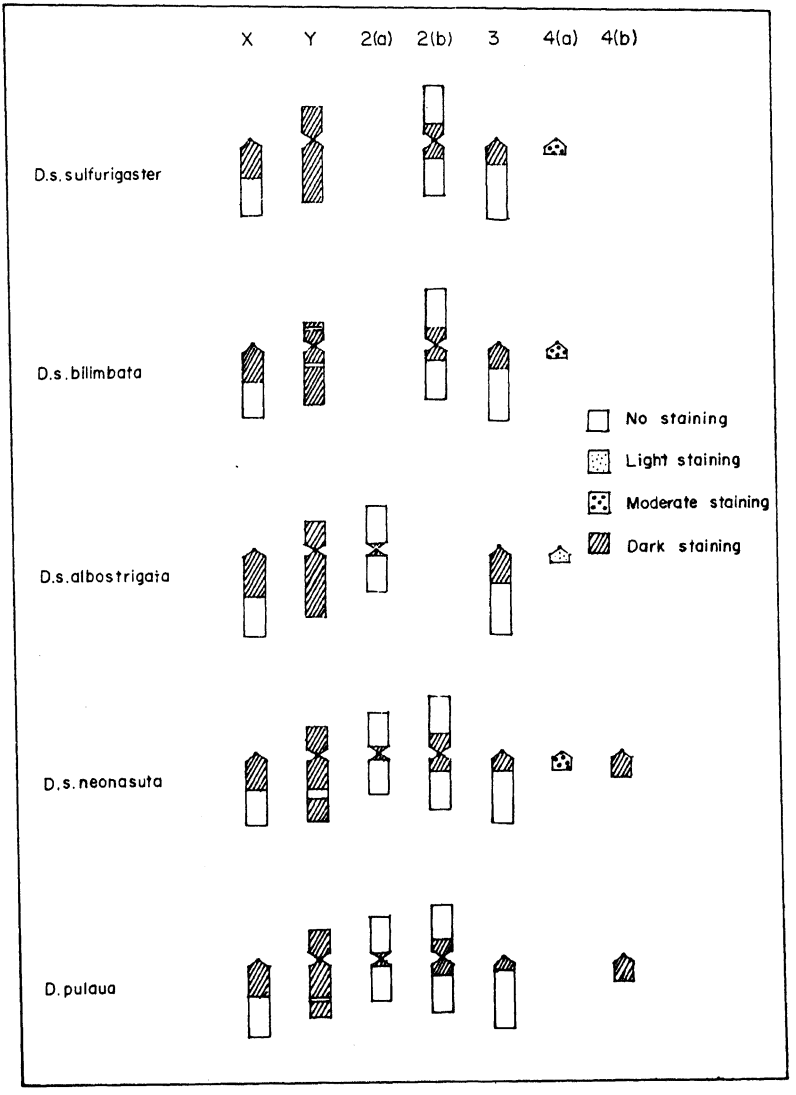

Figure 1. Metaphase chromosomes of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* as seen after C-banding.

heterochromatin regions recognized by C-banding. These C-band regions fluor uniformly in all chromosomes with the exception of chromosome Y. The diffe tiation of the heterochromatin of the Y chromosome is interesting. The Y chro some of *D.s. sulfurigaster* shows 3 Q-banded regions, of which one is lighter others; while in *D.s. bilimbata* two brightly fluorescing segments are seen. In *albostrigata* the short arm of the Y chromosome is brighter than the long arm. Th chromosome of *D.s. neonasuta* has revealed a clear linear differentiation in fluorescing regions separated by non-fluorescing ones. Differential fluores regions are also seen in the Y chromosome of *D. pulaua*. At the outset it can be that no two forms of the orbital sheen complex of *Drosophila* have Y chromosc which have a similar response to A-T specific dye, namely Quinacrine. differences reported for the chromosome 2 and the dot chromosomes, after banding, among the members under study, are very much distinct after Q-band

Thus, the karyotypic differentiation within the orbital sheen complex of *Droso* has occurred and it appears to be associated with changes in the heterochromati its chromosomes.

3.2 *Satellite DNA*

A molecular dimension of heterochromatin was provided when *in situ* hybridizat showed that highly repeated DNA sequences were predominantly located in form of chromatin (Pardue and Gall 1970; Jones and Robertson 1970). In the ligh notable differences in the amount and distribution of heterochromatin in metap chromosomes of the members of the orbital sheen complex, the satellite DNA these forms were analysed. The DNA extracted from the larval neural gangli these members were analysed by CsCl density gradient centrifugation and buoyant density profiles are shown in figure 3. Varying quantities of A-T satellite DNA is seen in different members under study. No two profiles resemble another. Four distinct A-T rich satellite fractions are seen in *D.s. sulfurigaster* (1· 1·661, 1·665 and 1·675 g/cm^3). In *D.s. bilimbata*, even though the quantum of sate DNA is more than that in *D.s. sulfurigaster*, the number of clearly separable sate fractions were only two. On the other hand, in *D.s. albostrigata*, *D.s. neonasuta* *D. pulaua*, the amount of satellite DNA is less and also separation is not as clear is in other forms. These preliminary studies on satellite DNA do demonstrate differences among these closely related forms of *Drosophila*.

Perusal of literature reveals that there exist conflicting reports with regard to role of heterochromatin and satellite DNA in evolution (Miklos *et al* 1980; Mi and Gill 1981). In view of these, it is premature to say anything about the impac differences in heterochromatin and satellite DNA on the evolutionary biology of orbital sheen complex of *Drosophila*.

3.3 *Population fitness*

Differential reproduction is the essence of Neo Darwinian natural selection. biological success of a species population can be measured in terms of its rep ductive success in relation to other populations. It is a cumulative assessmen fecundity, rate of development, viability and adaptedness. Each one of these c

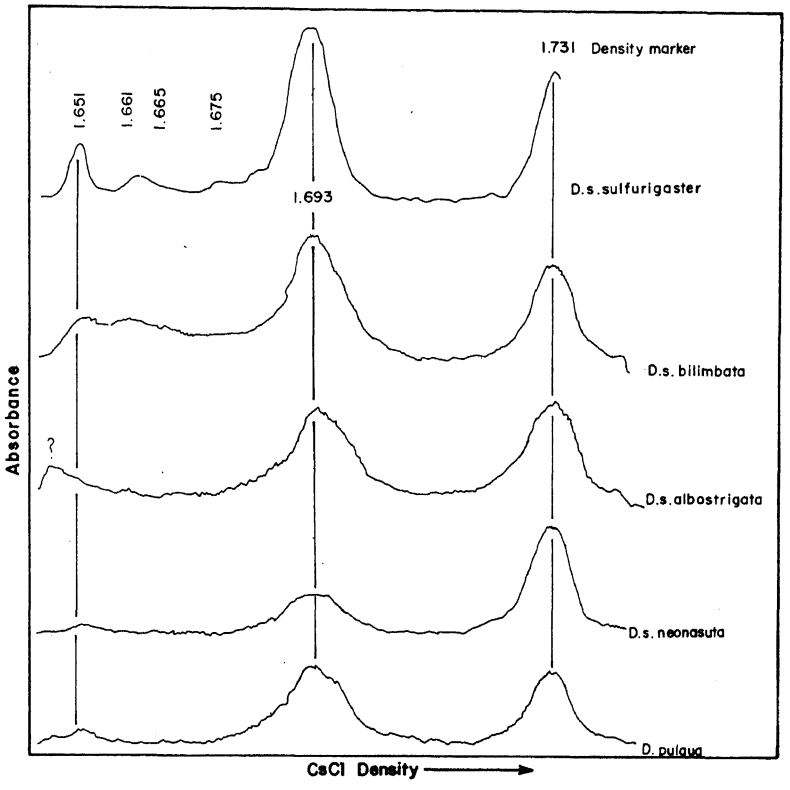

Figure 3. Density profile of DNA (from larval brains) of 5 strains of the orbital sheen complex of *nasuta* subgroup, after analytical CsCl equilibrium/density gradient centrifugation. The densities of the various DNA fractions were calculated relatively to *Micrococcus lysodeictius* according to the method of Szybalski (1968).

ponents contribute to the overall reproductive performance of a population. The differences in the reproductive abilities of different populations reflect the extent of fitness divergence and hence the underlying genetic differences between the concerned populations.

The fitness phenotypes of the members of the orbital sheen complex of *Drosophila* were compared by means of assessing certain parameters of fitness (tables 2 and 3). Of the 5 members under study, *D.s. neonasuta* was more fecund and also its eggs were more viable than others. The eggs of *D.s. albostrigata* had the fastest rate of egg to adult rate of development while it had the least degree of fecundity. *D.s. sulfurigaster* had the slowest rate of development while the eggs of *D.s. bilimbata* had the least degree of viability.

Populations of organisms must live and reproduce in order to be designated 'adapted'. Adaptedness refers to the ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment (Dobzhansky 1968). Four components of adaptedness namely population size, productivity, mortality and flies per bottle were estimated and the mean values of these are set forth in table 3. For all the components of adaptedness, *D.s. neonasuta* tops the list with

Table 2. Three pre-adult parameters of fitness, namely fecundity, egg to adult rate of development and egg to adult viability of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila.*

Strains	Fecundity (eggs/day/ individual)	Mean Developmental time (days)	Egg to adult viability. (for 500 eggs)
D.s. sulfurigaster	7·23	16·42 ± 0·08	148 (29·60%)
D.s. bilimbata	4·67	13·46 ± 0·14	83 (16·60%)
D.s. albostrigata	4·30	11·79 ± 0·12	185 (37%)
D.s. neonasuta	12·34	13·14 ± 0·09	303 (60·60%)
D. pulaua	7·82	15·17 ± 0·14	271 (54·20%)

Table 3. Mean values of population size, productivity, mortality and flies per bottle—4 components of adaptedness recorded in 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila.*

Strains	Population size	Productivity	Mortality	Flies per bottle
D.s. sulfurigaster	112·95 ± 4·21	93·87 ± 6·06	64·77 ± 6·37	32·86 ± 1·22
D.s. bilimbata	123·43 ± 7·85	82·78 ± 5·56	62·78 ± 3·70	35·91 ± 2·28
D.s. albostrigata	132·68 ± 7·31	109·45 ± 5·33	84·48 ± 8·26	38·60 ± 2·13
D.s. neonasuta	243·29 ± 11·37	146·38 ± 6·12	115·35 ± 5·10	70·78 ± 3·33
D. pulaua	133·89 ± 6·06	109·95 ± 8·82	82·37 ± 7·47	38·95 ± 1·53

significantly more values than those of others. The ranking is as follows: *D.s. neonasuta* > *D. pulaua* > *D.s. albostrigata* > *D.s. bilimbata* > *D.s. sulfurigaster.*

3.4 *Ecogenetic divergence*

Although many authors regard animal speciation as primarily the development of reproductive isolating mechanisms, Parsons (1981) has argued that an ability to use the resources of the environment in such a way as to be protected against niche competitors (Mayr 1977) is an equally, if not more important component. For a coherent evolutionary analysis of species and populations, an integration of genetics at the levels of populations and ecology is needed (Ushakumari and Ranganath 1986b). *Drosophila* has been used as a representative system to understand genetic basis of ecological differentiation (Parsons and Spence 1981; Powell and Andjelkovic 1983; Taylor and Condra 1983; Ramachandra and Ranganath 1986). Parsons and Spence (1981) have shown resource utilization divergence among 6 closely related *Drosophila* species of *melanogaster* subgroup in the utilization of ethanol and acetic acid as energy sources. In the present study, 3 types of sugars, sucrose, glucose and fructose were used as different resources and the response of the 5 members of the orbital sheen complex to these different sugars was evaluated in terms of rate of development, viability and population size. Table 4 gives the mean rate of development in days of the 5 strains in different media. The average rate of development ranges between 13 and 17 days in the media with sucrose, 12–16 days in the media with glucose and 12–13 days in the media with fructose. Thus, there is not much of a difference in the egg to adult rate of development of the 5 strains under study in the

Table 4. Egg to adult rate of development in days (mean ± SE) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	14·49 ± 0·12	15·36 ± 0·14	13·93 ± 0·12
D.s. bilimbata	17·87 ± 0·10	12·16 ± 0·11	13·36 ± 0·07
D.s. albostrigata	13·85 ± 0·11	13·25 ± 0·19	12·04 ± 0·04
D.s. neonasuta	13·92 ± 0·11	12·56 ± 0·12	12·85 ± 0·11
D. pulaua	17·37 ± 0·25	16·66 ± 0·22	13·94 ± 0·20

Table 5. Egg to adult viability (for 500 eggs) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	121 (24·20%)	55 (11%)	116 (23·20%)
D.s. bilimbata	78 (15·60%)	119 (23·80%)	140 (28%)
D.s. albostrigata	106 (21·20%)	32 (6·40%)	25 (5%)
D.s. neonasuta	253 (50·60%)	230 (46%)	238 (47·60%)
D. pulaua	117 (23·40%)	80 (16%)	114 (22·80%)

Table 6. Average population size (mean of 4 replicates) of 5 strains of orbital sheen complex of *nasuta* subgroup of *Drosophila* in media with different sugars.

Strains	Sucrose	Glucose	Fructose
D.s. sulfurigaster	93·63 ± 2·33	64·14 ± 4·84	42·77 ± 3·96
D.s. bilimbata	89·22 ± 2·62	56·34 ± 3·02	58·50 ± 3·60
D.s. albostrigata	75·92 ± 5·27	34·97 ± 2·32	57·47 ± 1·34
D.s. neonasuta	188·13 ± 5·81	147·97 ± 6·64	73·82 ± 2·30
D. pulaua	75·98 ± 3·48	31·86 ± 2·51	68·88 ± 2·39

media with fructose, but the differential response of these strains to media either with sucrose or glucose is revealing. Similarly, the response of the eggs of any one strain to different media reveal that the eggs of *D.s. albostrigata* and *D.s. neonasuta* have completed egg to adult development within a span of 12–13 days in 3 types of media, while it was 12–17 days for the eggs of *D.s. bilimbata* and 13–17 days for the eggs of *D. pulaua.*

The findings on egg to adult viability of the 5 strains of *Drosophila* in different media is shown in table 5. In all the 3 types of media, eggs of *D.s. neonasuta* were found to be more viable than the eggs of other strains. The range of variation in the extent of viability in the media with sucrose was 15–50%, in the media with glucose it was 6–46% and in the media with fructose it was 5–47%. Intrastrain comparison shows that except *D.s. bilimbata*, remaining 4 strains had more viability in the media with sucrose than in other media, while the eggs of *D.s. bilimbata* were more viable in the media with fructose and it had the least viability in the media with sucrose.

In addition to these, the overall average population size maintained by these 5 strains in different media has been estimated and the same is given in table 6. *D.s. neonasuta* has emerged as the most successful strain in all the 3 types of media as

indicated by its highest mean values. *D.s. neonasuta* had a mean population size of 188, 147 and 73 in the media with sucrose, glucose and fructose respectively.

These studies with different sugars have exposed differential abilities of these strains to exploit the media with different sugars. This is an indication of inherent divergence of these strains to exploit a resource material present in the ambient system.

3.5 *Isozyme variations*

The electrophoretic revolution of the last two decades has proved that electrophoretic technique is a powerful tool to assess genetic divergence and/or identity between populations/races/species (Throckmorton 1977). Recently, Buth (1984) has critically evaluated the application of electrophoretic data in systematic studies. Ramesh and Rajasekarasetty (1980) have analysed the genetic variability at loci concerned with acid phosphatase, alkaline phosphatase, α-esterase, β-esterase, α-glycerophosphate dehydrogenase and tetrazolium oxidase in these 5 members of the orbital sheen complex of *Drosophila*. The summary of their findings in terms of genetic identity and genetic distance among these members are compiled in table 7. These values suggest the level of genetic differentiation at these loci studied in the *Drosophila* members under study. The maximum genetic distance of 0·705 was noticed between *D.s. neonasuta* and *D. pulaua*, while the least genetic distance of 0·378 was between *D.s. albostrigata* and *D.s. sulfurigaster*.

4. Conclusion

These morphologically similar and taxonomically and phylogenetically parsimonious members of the orbital sheen complex of *Drosophila* have striking differences in their karyotypic organization, satellite DNA, fitness phenotypes, ecogenetic divergence and isozyme variations. Evolutionary divergence is a dynamic process and each one of the above said features represents different facets of differentiation. Further, it is difficult to establish and to universalise the correlations between different phenotypes of evolving populations. This is especially important, when considering the fauna on a world wide scale, after all, evolutionary patterns, rates and pressures should not be expected to produce uniform results at all places, at all times and in all aspects (Wheeler 1981). Therefore, the genetic history and evolutionary fate of each population is unique to itself and it is beyond the range of predictability.

Table 7. Values of genetic identity, Ixy (above diagonal separation) and distance, Dxy (below diagonal separation) estimated among different members of the orbital sheen complex of the *nasuta* subgroup of *Drosophila* (Ramesh and Rajasekarasetty 1980).

DXY \ IXY	*D. pulaua*	*D.s. neonasuta*	*D.s. sulfurigaster*	*D.s. bilimbata*	*D.s. albostrigata*
D. pulaua	—	0·494	0·571	0·639	0·678
D.s. neonasuta	0·705	—	0·549	0·612	0·573
D.s. sulfurigaster	0·561	0·600	—	0·574	0·685
D.s. bilimbata	0·448	0·491	0·555	—	0·680
D.s. albostrigata	0·389	0·557	0·378	0·386	—

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Chromosomal basis of raciation in *Drosophila*: A study with *Drosophila nasuta* and *Drosophila albomicana*

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Abstract. *Drosophila nasuta nasuta* (2n = 8) and *Drosophila nasuta albomicana* (2n = 6), a pair of cross fertile races have conspicuous karyotypic divergence and symptoms of post-mating isolating mechanisms. Hybridization of these races followed by hybrid maintenance and hybrid recombination has resulted in the evolution of two novel races with new combinations of parental chromosomes referred to as Cytoraces I and II and this has illustrated an interesting aspect of karyotypic differentiation under laboratory conditions.

Keywords. *Drosophila*; chromosomal races; hybridization; evolution.

1. Introduction

Drosophila nasuta and *D. albomicana* are sibling members of the *nasuta* subgroup of the *immigrans* species group of *Drosophila*. In this article an attempt has been made to review the chromosomal basis of their differentiation.

D. nasuta was described by Lamb (1914) and the type specimens were collected from Seychelles Islands. Duda (1923) described *D. albomicana* from Paroe, Formosa and it was morphologically similar to *D. nasuta*. Wilson *et al* (1969) have made extensive cytogenetic and hybridization studies of the members of the *nasuta* subgroup and they were able to solve the status of some members. But, *D. nasuta* (*sensu strictu*) was not available to Wilson *et al* (1969) and in the absence of this member they felt that 'in view of the great similarities between the species of this complex and the remarkable degree of speciation which has taken place in south-east Asia, it seems more reasonable to assume that the true *nasuta*, more than 3000 miles from the closest known *albomicans*, is still another species'.

Nirmala and Krishnamurthy (1971) reported a *D. nasuta* strain from Soundathi (south India) possessing 2n = 8. The karyotype of *D. nasuta* from Seychelles Islands, from where Lamb (1914) described it was analysed by Wakahama and Kitagawa (1972) and based on the similarities between Seychelles Islands and south Indian strains, they opined that Indian *D. nasuta* may be the same species or a closely related species of *D. nasuta sensu strictu*. This was followed by the findings of Ranganath *et al* (1974), wherein the hybridization experiments showed that Indian and the Seychelles Islands strains of *D. nasuta* are completely cross fertile and they are one and the same species.

Hybridization studies involving south Indian strains of *D. nasuta* with 2n = 8 and *D. albomicana* of Taiwan with 2n = 6 showed that these two so called species constitute open genetic systems (Nirmala and Krishnamurthy 1972). This was confirmed by the studies of Ranganath *et al* (1974) that both the Indian as well as the Seychelles Islands strains of *D. nasuta* and the Taiwan and the Okinawan strains of *D. albomicana* are cross fertile. In view of this open genetic systems and cross fertility

between *D. nasuta* and *D. albomicana* and taking into cognizance the differences in their karyotypes, they have been treated as chromosomal races and called *D. n. nasuta* (2n = 8) and *D. n. albomicana* (2n = 6) (Nirmala and Krishnamurthy 1972; Ranganath *et al* 1974).

With regard to geographic distribution, *D. nasuta* is wide spread. It has been reported from Mombusa, Kenya; Tananarive Madagascar; Mahe, Seychelles; Mauritius Island; Reunion Island; Kandy, Sri Lanka (Kitagawa *et al* 1982), Mysore and other localities of India (Ranganath and Krishnamurthy 1972; Reddy 1973; Siddaveere Gowda *et al* 1977; Prakash 1980; Muniappa 1982; Gai 1985); and west coast of Africa (David and Tsacas 1980). *D. albomicans* is reported from several Japanese Islands and Malaysia (Kitagawa *et al* 1982); Thailand (cf. Wilson *et al* 1969); Taiwan (Lin *et al* 1977); and Shillong (Singh 1977). All these reports indicate that *D. nasuta* and *D. albomicana* are allopatric.

2. Direction of evolution

The direction of evolution between the two races, viz *D. n. nasuta* and *D. n. albomicana* was discussed by taking into consideration their karyotypes and mating preference. Wilson *et al* (1969), Wakahama *et al* (1971), Nirmala and Krishnamurthy (1972) and Ranganath *et al* (1974) have argued and showed that the karyotype of *D. n. albomicana* (2n = 6) is derived from that of *D. n. nasuta* (2n = 8). The evolutionary event that is responsible for this change was the centric fusion between chromosome 3 and the sex chromosomes of *D. n. nasuta* to produce a metacentric chromosome of *D. n. albomicana* (X3; Y3) and this has reduced the diploid number from 2n = 8 to 2n = 6. Recently, Ranganath and Hagele (1981), while discussing the karyotypic evolution in the *nasuta* subgroup have demonstrated that the karyotype of *D. n. albomicana* is the recent product in the group and also illustrated it as a product of karyotypic ortho-selection involving successive centric fusions. In view of this karyotypic phylogeny, *D. n. nasuta* is ancestral to *D. n. albomicana.*

The direction of evolutionary lineage can also be discussed by studying the mating preference between closely related forms. Ramachandra and Ranganath (1987) have analysed the mating preference between *D. n. nasuta* and *D. n. albomicana.* In no choice experiments, when there was no choice in the cross involving males of one race with the females of the other, all the crosses were successful. In multiple choice experiments both the females and males of each race were placed together. Here, all the possible matings like homogamic, heterogamic and one male mating with both the females were recorded. However, homogamic and matings of males of *D. n. nasuta* with the females of both the races were more frequent compared to other matings. 'Female choice' experiments showed that both *nasuta* (ancestral) and *albomicana* (derived) females have chosen the males of *nasuta* (ancestral) to the males of the derived race, viz *D. n. albomicana.* Further, even in the male choice experiments, wherein males of one race were given the choice to select between the females of both the races, the males of these two races have preferred the females of *D. n. nasuta* more than that of *D. n. albomicana.* These observations fit into the hypothesis of Kaneshiro (1976) which states that females of ancestral species strongly discriminate against males of the derived species, while females of the derived species readily accept the males of the ancestral species.

Thus there is good correlation between the findings of the mating preference xperiments and the karyotypic phylogeny mentioned earlier. In view of these, it can e safely mentioned that the direction of evolution was from *D. n. nasuta* to *). n. albomicana.*

. Karyotypic organization

The diploid chromosome complement of *D. n. nasuta* is 2n = 8 (figure 1A, B) (Nirmala nd Krishnamurthy 1971; Wakahama and Kitagawa 1972; Ranganath 1978; Ranganath *et al* 1974; Lakhotia and Kumar 1979). It includes one pair of metacentric chromosomes (chromosome 2), two pairs of acrocentrics (chromosome X and) and a pair of small basic dots (chromosome 4). In males one of the X chromosome s replaced by a submetacentric Y chromosome. The metaphase chromosome complement of *D. n. albomicana* (2n = 6; figure 1C, D) consists of two pairs of metacentric chromosomes (chromosome 2 and X3, X3 or X3, Y3) and a pair of long dots Wilson *et al* 1969; Nirmala and Krishnamurthy 1972; Ranganath 1978).

Heterochromatin distribution and differentiation in metaphase chromosomes of hese two races, *D. n. nasuta* and *D. n. albomicana* were studied by Ranganath and

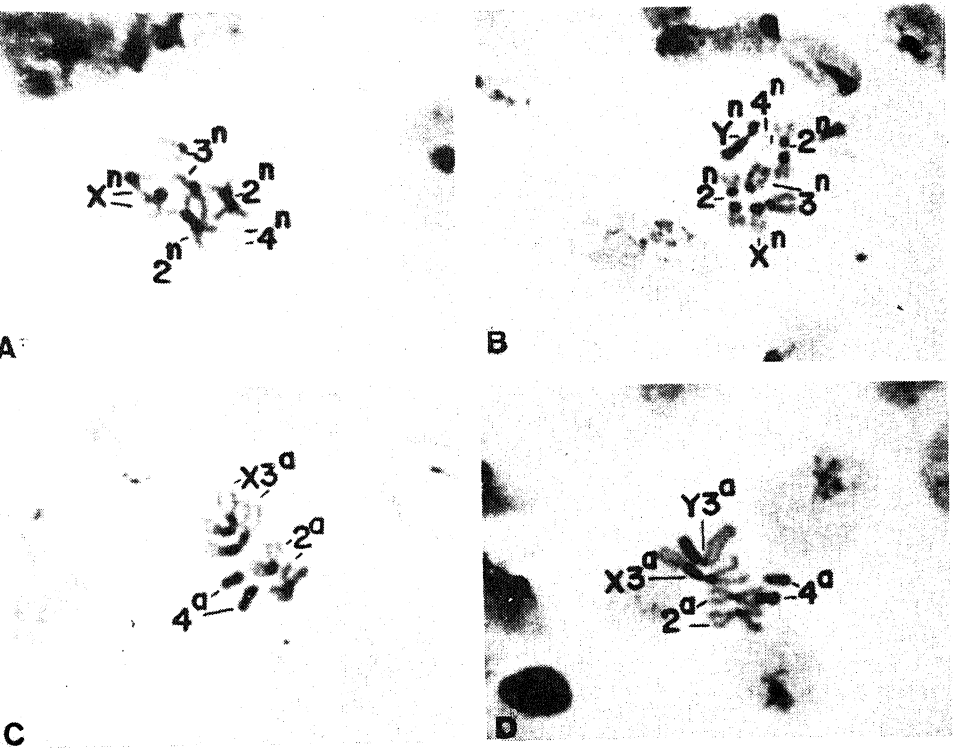

Figure 1. C-banded metaphase chromosomes of *D. n. nasuta* (2n = 8) female (**A**) and male (**B**); and of *D. n. albomicana* (2n = 6) female (**C**) and male (**D**). Chromosomes are designated with superscripts n for the chromosomes of *D. n. nasuta* and a for the chromosomes of *D. n. albomicana.*

Hagele (1982) and Ranganath *et al* (1982) by way of C-, N- and Q-banding methods. It has been demonstrated that between the homologous metaphase chromosomes of these races there exist length differences and this is mainly due to differences in the size of heterochromatin blocks. Significant differences are seen in chromosome 2 and 4 of these races. The N-banding studies of Ranganath and Hagele (1982) have revealed that the Y chromosomes of both races and the chromosome 4 of *D. n. albomicana* possess heterochromatin sections which behaves differently from those of other centric and pericentric heterochromatin regions. The AT-richness of heterochromatin in these *Drosophila* races was shown by its bright quinacrine fluorescence (Ranganath *et al* 1982). In the metaphase chromosomes of *D. n. nasuta* and *D. n. albomicana*, the regions of bright fluorescence coincide with C-banded regions.

Ranganath *et al* (1982) have shown that the DNA of *D. n. nasuta* has only one major AT-rich satellite fraction with a density of 1·664 g/cm^3 and it amounts to about 7 to 8% of the total DNA. On the other hand, *D. n. albomicana* had atleast 3 different AT-rich satellites with densities of 1·674 g/cm^3, 1·665 g/cm^3 and 1·661 g/cm^3 and these comprise 28–30% of its total DNA. *In situ* hybridizations to metaphase and polytene chromosomes have shown that cross hybridizations were successful and hence Ranganath *et al* (1982) have felt that there are homologous satellite DNA sequences in the heterochromatin areas of the chromosomes of *D. n. nasuta* and *D. n. albomicana* and the satellite DNAs of these races differ quantitatively rather than in quality.

Attempts have been made by Hagele and Ranganath (1983) to localize NORs by way of combined acid treatments and Ag-AS techniques. These studies suggest that the NORs in these races are present on heterochromatic Y-chromosomes and on chromosome 4 and this appears to be a rare combination in *Drosophila.*

In polytene cells, in both the races there are 4 long arms (X, 2L, 2R and 3) and a short arm (4) (Ranganath and Krishnamurthy 1973–74; Wilson *et al* 1969). In the F_1 hybrids of *D. n. nasuta* and *D. n. albomicana* fixed inversion differences were seen in 2L and 3 chromosome arms, while 2R and X were devoid of such differences (Ranganath 1978; Rajasekarasetty *et al* 1978, 1980). In addition to these, significant differences between the short chromosome arm 4 of these races have been demonstrated by Hagele and Ranganath (1982). The metaphase chromosome 4 of *D. n. albomicana* is nearly 5 times larger than that of *D. n. nasuta.* But in polytene cells, it is much shorter than that of *D. n. nasuta.* In the F_1 polytene cells, these homologous chromosomes do not pair and they appear as twins in contrast to other arms. Hagele and Ranganath (1982) have illustrated that this is due to a duplication, an inversion and a massive amount of heterochromatin in the chromosome 4 of *D. n. albomicana* and this makes it different from that of its homologue in *D. n. nasuta* which lacks these features.

4. Interracial hybridization

The F_1 hybrids of the cross between *D. n. nasuta* (2n = 8) and *D. n. albomicana* (2n = 6) had 2n = 7. Inspite of the above narrated differences between these races in their karyotypic organization, the F_1 and the succeeding hybrid progeny were fertile. It was of interest to know the dynamics of chromosome segregation during gametogenesis in the F_1 hybrids and its impact on the fertility of the next generation.

Ranganath and Krishnamurthy (1981) have probed this facet of hybrids and their findings indicate that F_1 males produce 6 different types of sperms. Of these 39% had the normal haploid chromosome complement while the remaining 61% were aneuploids for chromosomes X or 3. On the other hand, the F_1 female produced only two types of eggs with haploid quantum of chromosomes and the aneuploid eggs were not seen. Fertility test on F_2 and backcross progeny have shown that males are more often sterile than females. Tested males of 177 out of 400 were sterile and 14 out of 400 females analysed were sterile. Thus, the F_1s produce both fertile and sterile F_2 individuals. The birth of the succeeding generations is taken care of by the fertile individuals and hence it is possible to maintain the hybrid populations of *D. n. nasuta* and *D. n. albomicana* for any number of generations.

The chromosomal basis of coadaptation analysed by Ranganath (1978) showed that the F_1 hybrids of *D. n. nasuta* and *D. n. albomicana* were heterotic for certain parameters of fitness and there was a hybrid breakdown in F_2. This was taken as an evidence to demonstrate the divergence in the genetic constitution of these races.

The analysis of the karyotypes of the F_2 progeny and of the succeeding generations of the cross between *D. n. nasuta* and *D. n. albomicana* showed that the hybrid populations, karyotype-wise are extremely heterogeneous with different types of karyotypes. Ranganath (1978) and Rajasekarasetty *et al* (1979) have termed this as 'karyotypic mosaicism', wherein one can see different chromosome combinations other than the parental and of the F_1 hybrids.

Recently, Ramachandra and Ranganath (1986) have made a systematic assessment of fate of different parental chromosomes of *D. n. nasuta* and *D. n. albomicana* in their hybrid populations. They have recorded that the karyotypic mosaicism present in the hybrid populations declines over generations and during this process there was selective elimination of some parental chromosomes, while some other chromosomes were retained. Finally by F_{20} the karyotypic variability disappears and karyotypically a stable hybrid population evolves. Such an event has led to the emergence of two new strains of hybrid populations, which differ from their parents as to the karyotypic composition and hence these newly derived lines have been called Cytoraces (Ramachandra and Ranganath 1986). The salient features of this interesting experiment are as follows:

The hybridization between males of *D. n. nasuta* and females of *D. n. albomicana* has resulted in the evolution of a hybrid population with new karyotypic combination and this has been called Cytorace I (figure 2A, B). The males of Cytorace I had $2n=7$ (2^a2^n; Y^nX3^a; 3^n; 4^n4^n) and females had $2n=6$ (2^a2^n; $X3^aX3^a$; 4^n4^n). Of the 13 chromosomes of Cytorace I, 8 were of *nasuta* parent and 5 were of *albomicana* parent. The superscripts in the figures denote the parent from which that particular chromosome was inherited—n = *D. n. nasuta*; a = *D. n. albomicana.* Inspite of the difference in the diploid number of chromosomes in males and females, Cytorace I breeds true. The chromosomal mechanism underlying this true breeding nature of Cytorace I has been illustrated by Ramachandra and Ranganath (1986). Thus, this karyotypic duality between males and females of a strain with its true breeding character is a unique report and first of its kind in *Drosophila.*

The hybrid population of the cross between males of *D. n. albomicana* and females of *D. n. nasuta,* after passing through an ephimeral phase of karyotypic mosaicism has attained karyotypically a stable state with males and females having $2n=6$. This hybrid population was called Cytorace II (figure 2C, D) (Ramachandra and

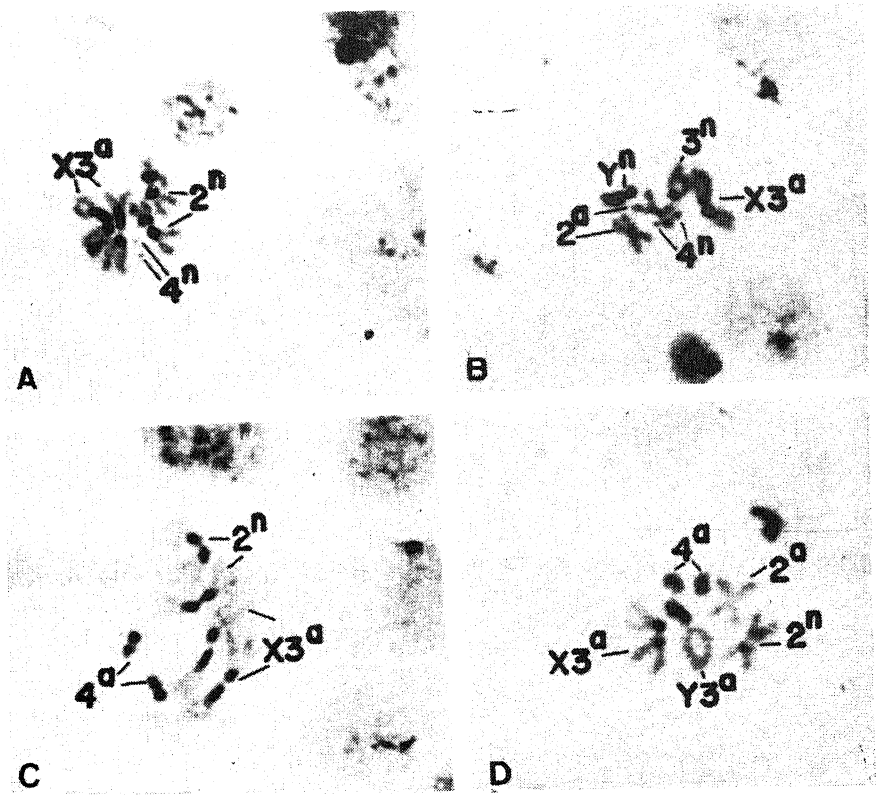

Figure 2. C-banded metaphase chromosomes of Cytorace I: female 2n = 6 (**A**) and male 2n = 7 (**B**); and of Cytorace II: female 2n = 6 (**C**) and male 2n = 6 (**D**). Chromosomes are designated with superscripts n for the chromosomes of *D. n. nasuta* and a for the chromosomes of *D. n. albomicana.*

Ranganath 1986). The chromosomes that constituted the male karyotype were 2^n2^a, $X3^aY3^a$, 4^a4^a and similarly, of the female it was 2^n2^a; $X3^aX3^a$; 4^a4^a. Of these 12 chromosomes, 10 were of *albomicana* parent, while only two came from *nasuta* parent.

The comparative evaluation of the karyotypes of the parents and of the Cytoraces reveal that the Cytorace I is devoid of the X chromosome of *D. n. nasuta* and chromosome 4 of *D. n. albomicana.* Similarly, the Cytorace II is without the representation of chromosomes X, 3 and 4 of *D. n. nasuta.* These chromosomes which are not seen in the stabilized karyotypes of Cytoraces were found in their respective F_1 karyotypes and these have been eliminated in the succeeding hybrid generations. On the whole, the behaviour of dot chromosomes (chromosome 4) is very interesting. In each cross, there is a good correlation between the male parent of the cross and the dot chromosome that has been retained in the stabilized population. Male parents of Cytoraces I and II were *nasuta* and *albomicana* respectively. Correspondingly, the Cytorace I had the dot chromosomes of *nasuta* only, while the Cytorace II had the dot chromosomes of *albomicana* only. This type of affinity between the dot and Y chromosomes of these races may be underlined by the presence of NORs in these two chromosomes of these races. It is also evident in the karyotypes of these cyto-

races, that there is selective preference for the parental combinations of the dot chr mosomes over the hybrid association. This may be due to 'incompatability' betwe these parental chromosomes in view of significant structural changes involved in t evolution of the dot chromosomes of *D. n. albomicana* as discussed earlier.

The karyotypes of Cytoraces I and II are the products of elements derived fro two different races, viz *D. n. nasuta* and *D. n. albomicana.* The restructuring of t karyotypes has resulted in significant differences in the quantum of heterochromat between the parental races and the newly evolved Cytoraces (Ramachandra a Ranganath 1986). Of these 4 races, the individuals of Cytorace I had the lowe amount of heterochromatin, while those of Cytorace II had the highest quantum C-band DNA. Hence, these have been referred to as heterochromatin 'poor' a 'rich' strains respectively.

The extent of divergence between the parental races and the newly evolved Cyt races has been shown by way of demonstrating the differences in their fitness phen types (Ramachandra N B and Ranganath H A, unpublished results). In brief, t sequence for 4 different parameters of fitness is as follows: for fecundity: Cytora I = *D. n. nasuta* = Cytorace II > *D. n. albomicana*; for rate of development: Cytora I > Cytorace II > *D. n. albomicana* > *D. n. nasuta*; for viability: *D. n. nasuta* = Cytora II > Cytorace I = *D. n. albomicana*; and for adaptedness: *D. n. albomicana* *D. n. nasuta* > Cytorace II > Cytorace I. These differences do suggest the underlyi genetic differences between these races.

5. Conclusion

Interracial hybridization between two coadapted gene pools, viz *D. n. nasuta* a *D. n. albomicana* has resulted in the evolution of two new races. In a way, in t instance, hybridization has acted as an 'evolutionary catalyst' in terms Dobzhansky *et al* (1976). The karyotypes of these cytoraces are new in compositi and each one of them have inherited chromosomes from both the parents.

Recently, Templeton (1981) has argued that hybridization followed by the proc ction of unstable hybrids, inbreeding and hybrid breakdown may result in a form natural selection favouring those F_2 and later individuals that have better viabil and fertility. This may result in the formation of a new rare recombinant class genotype. Templeton (1981) has called this as 'transilience mode of speciation'. T karyotypes of the parental races, viz *D. n. nasuta* and *D. n. albomicana* as well those of newly evolved races followed by hybridization, viz Cytoraces I and represent two distinct phases of karyotypic stability. These two phases were sep rated by a transient phase of karyotypic instability due to karyotypic mosaicism the hybrid populations. Thus, this is an interesting facet of evolutionary process, 'karyotypic differentiation' followed by hybridization under laboratory conditio and these sequence of events as reported by Ramachandra and Ranganath (1986) into the 'transilience mode of speciation' proposed by Templeton (1981).

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Importance of Karyology in aphid taxonomy

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Abstract. Cytological parameters are nowadays being increasingly and intelligently used in solving the taxonomic problems of dubious species. Aphids', in certain cases atleast, taxonomy is not stabilised at the species as well as at the subgeneric level. It is because of the usual occurrence of race or biotype in natural population. Occurrence of such taxonomic categories are possibly due to chromosomal rearrangements or otherwise that resulted from the association with a host plant, as evidenced in a few species, viz, *Myzus persicae*, *Brachycaudus helichrysi*, *Tuberolachnus salignus* etc. Several ecogeophysical factors are operative in establishing chromosomally distinguished races which demand the attention of taxonomists for further taxonomic evaluation of the concerned species. Attempt is therefore made to discuss the rationale of using the cytological informations in the identification of infraspecific categories of aphids.

Keywords. Aphid; biotype; karyology.

Aphids among the homopterans are possibly of special interest to the cytologists because of extensive polymorphism as well as complicated mode of life cycle (cyclical parthenogenesis) within the group itself. Furthermore, confusion over the taxonomic identity of the species of economic importance has arisen because several perfectly good biological species are very similar in their gross morphology. Such puzzling problems attracted the aphidologists to verify them cytotaxonomically. Their study includes the cytogenetical aspects of aphids that help the taxonomists to separate the closely related species of dubious taxonomic status by supplementing them with the evidences from karyotypical differences. Valuable bulk of data is available from the extensive studies of chromosomal variation of different aphids of higher taxonomic categories that may provide a guideline in clarifying the natural relationship of species as well as their probable way of phylogeny.

The range of karyotypic variation among aphids is broad. Sometimes a single genus may include several karyotypes. On the contrary, distantly related species are also with identical karyotypes. The conventional way of comparing identical species, in respect of morphological features could be well supplemented by certain cytological parameters, viz diploid number, structure, karyometrical analysis, chromosome formula (CF) (Khuda-Bukhsh and Pal 1984; Das *et al* 1985). Therefore, in the field of aphid taxonomy cytological studies can be used as a convenient tool in which somatic chromosomes in prophase or metaphase, by squashing the young embryos, can be obtained without difficulty which enables to study the basic feature of karyotypes, viz diploid number (2n), actual length of chromosomes (μm), relative length ($R^1\%$), total complement length (TCL), CF etc.

Homopteran chromosomes are holocentric which is also shared by Heteroptera, Mallophaga, Anopleura, Dermaptera, probably Lepidoptera, some scorpions and some centipedes. Due to lack of primary point of reference, aphid chromosomes appear featureless, rod or dot shaped depending on the size, excepting the occasional

occurrence of conspicuous secondary constriction in conventional preparations. But by employing recently developed advanced methods (C and G-banding) now it is possible to include specific pattern of bands along the length of a chromosome and accordingly it can be identified from its counterparts.

Sexual morphs, which generally occur in nature at the advent of adverse conditions like cold climate or can be produced artificially in glass-house from the cultures of virginoparae in late spring by inducing short photoperiod are indispensible for studying the meiotic stages, which is less touched by aphid cytologists in comparison to somatic chromosomes. For this practical reason present observations are made from the study on the somatic chromosomes.

Clonal variability of an aphid species in respect of life cycle, food specialization and morphology even in restricted ecological niche, often presents complicated situation to taxonomists. This may be evidenced from the following studies.

Myzus persicae (Sulzer) collected from two different host plants, *Nicotiana tabacum* (Solanaceae) and *Rhaphanus sativus* (Cruciferae) at different seasons and different localities of north east India which biologically might vary (Eastop 1973) has been found to vary karyotypically (tables 1 and 2). Sixty eight per cent of 79 totally examined plates were with $2n=12$ which is also supported by the earlier reports (Blackman and Takada 1977; Kulkarni and Kacker 1981; Khuda-Bukhsh and Pal 1986; Kuznetsova 1969; Sun and Robinson 1966). Other aneuploids with $2n=8$ and 13 may be due to dissociation, fusion or translocation being robertsonian fusion as the main operative force. In some cases occurrence of clear structural heterozygosity was noticed in one of the X-chromosomes of the diploid complement.

From tables 1 and 2 it is apparent that the populations collected from two different host plants do not significantly vary karyometrically but seasonal variation casts a significant change in karyotype. In both the clones chromosome formula stands $n=2M+2S+2S^v$ but in response to seasonal variation, in clone-A one additional type of chromosome (i.e. long type; L) appears and accordingly the chromosome formula comes as $n=1L+1M+2S+2S^v$. In clone-B though CF remains the same but marked distinction could be observed between the second and fourth pairs of chromosomes.

Another clone collected from *R. sativus* during winter and summer from the plains

Table 1. Karyometrics of the haploid set of chromosomes of *M. persicae* from *N. tabacum*.

Locality	Season	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
Darjeeling (c 70000 ft.)	May	5·97 ± 0·08	29·70	20·10	$2M+2S+2S^v$
		(6·30 ± 0·09)	(31·03)	(20·30)	
		5·77 ± 0·07	28·71		
		(5·80 ± 0·09)	(28·57)		
		(3·56 ± 0·11)	17·71		
		(3·48 ± 0·07)	(17·14)		
	December	2·56 ± 0·09	12·74		$(1L+1M+2S+2S^v)$
		(2·50 ± 0·07)	(12·32)		
		1·23 ± 0·08	6·12		
		(1·32 ± 0·08)	(6·50)		
		1·01 ± 0·05	5·02		
		(0·90 ± 0·08)	(4·43)		

Table 2. Karyometrics of the haploid set of chromosomes of *M. persicae* from *R. sativus*.

Locality	Season	Actual length (μm ± SE)	Relative length (R^1%)	TCL	CF
Darjeeling (*c* 7000 ft.)	May	6·09 ± 1·51	29·91		
		(4·82 ± 0·09)	(25·06)		
		5·91 ± 1·03	29·03		
		(4·05 ± 1·20)	(21·81)		2M + 2S + 2S^v.
	December	3·56 ± 0·08	17·49		
		(3·55 ± 1·38)	(19·12)	20·36	
		2·62 ± 1·21	12·82	(18·57)	
		(3·47 ± 0·07)	(18·69)		(2M + 2S + 2S^v)
		1·21 ± 0·05	5·94		
		(1·48 ± 0·08)	(7·97)		
		0·98 ± 0·08	4·81		
		(1·20 ± 1·02)	(5·28)		

Clone-B; Populations 1 and 2 (in parentheses).

Table 3. Karyometrics of the haploid set of chromosomes of *M. persicae* from *R. sativus* showing n = 4.

Season	Actual length (μm ± SE)	Relative length (R^1%)	TCL	CF
Summer	1·47 ± 0·33	35·08.		
	(5·84 ± 0·17)	(37·77)		
	1·10 ± 0·32	26·25	4·19	
	(4·41 ± 0·19)	(28·53)		1L + 2M + 1S in both the cases
Winter	0·91 ± 0·30	21·72	(15·46)	
	(3·21 ± 0·13)	(20·76)		
	0·71 ± 0·13	16·95		
	(2·00 ± 0·13)	(12·94)		

Clone-C; Populations 1 and 2 (in parentheses).

of West Bengal (around Calcutta) exhibited 2n = 8 (table 3) which might be due to fusion of 1st and 6th and 3rd and 5th pairs of chromosomes of the normal karyotypes (tables 1 and 2).

Here the chromosome formula stands as n = 1L + 2M + 1S, very much akin to *Aphis* group, for which the preserved material were reexamined and the identification remained valid. However, in this clone seasonal variation does not hold any good distinctive criterion. Though the actual length of the chromosome seems to be longer in size yet it may be considered due to relative condensation of chromosomes during cell cycle.

Therefore, at least in case of *M. persicae*, it is established that in response to host plant, season or geographical distribution, several chromosomal races may be in nature which might have reflected on the morphology and/or biology of the species concerned. Such instances could be encountered with *Rhopalosiphum maidis* (Chattopadhyay *et al* 1982).

An year round cytological survey on a macrosiphine aphid, *Brachycaudus helichrysi* also speaks in favour of the existence of several chromosomal races in natural

populations. *B. helichrysi* collected from *Artemisia vulgaris* (Compositae) showed 2n = 12. Six homomorphic pairs with n = 2M + 2S + 2S^v, 4·65 μm and 1·00 μm being the length of the chromosome from longest to shortest. But in the sample collected from *Brassica napus* (Cruciferae), the karyology varied to a great extent (table 4).

Here an additional type (long type; L) of chromosome appears indicating 2n = 10 with chromosome formula as n = 1L + 1M + 2S + 1S^v. Another distinctive criteria between the two populations is that the maximum and minimum size difference exists between the 2nd and 3rd pair and 5th and 6th pair of chromosomes in the population from *A. vulgaris* which measures 5·32 and 3·06 R^1%, respectively in contrast to 8·90 and 4·55 R^1% size difference between the 2nd and 3rd and 4th and 5th pair of chromosomes respectively, in the sample collected from *B. napus*. We encountered similar incidence while working with a lachnine species, *Tuberolachnus salignus*. Populations established at distantly related host plants markedly varied karyotypically. Populations collected from *Pinus cassia* (Pinaceae) exhibited 2n = 14, (i) n = 1M + 5S + 1S^v and in some instances (ii) 6S + 1S^v. However, the M-type chromosome of the former can be omitted since it remains in the boundary line (R^1% – 20·14). So the CF is n = 6S + 1S^v (table 5). But the populations collected from *Salix babylonica* (Salicaceae) presented a different picture with 2n = 22; n = 4S + 7S^v which may arise as a result of fragmentation of the chromosomes (table 6). Thus it seems that host plant variation affects the karyotype of the species.

Sometimes a special problem arises in determining the exact identity of an aphid when one unfortunately deals with a mixed population of two or more species cohabiting the same host plant. To exemplify the situation reference is made to rose infesting aphids *Macrosiphum rosae* (Linn) and *Sitobion rosaeiformis* (Das). Very often they are found in close, in one and the same colony and live colours of the two species overlap to a great extent from greenish to pinkish. In this connection it may be pointed out that *M. rosae* has world wide distribution although entirely absent in eastern Asia, Japan in particular (Eastop 1966; Blackman and Eastop 1984). However, Eastop (1966) opines that *M. rosae* has been replaced in far eastern countries by several species including *Sitobion ibarae* (Mats.) and *S. rosaeiformis*. Simultaneous find of both *M. rosae* and *S. rosaeiformis* in India does not substantiate Eastop's contention. But from our cytological studies these two confusing species

Table 4. Karyometrics of the haploid set of chromosomes of *B. helichrysi*.

Host plants	Actual length (μm ± SE)	Relative length (R^1%)	Diploid No (2n)	TCL	CF
	4·65 ± 0·07	28·40			
	(3·75 ± 1·90)	(32·75)	12	16·37	n = 2M + 2S + 2S^v
	3·87 ± 0·07	23·64			
A. vulgaris	(3·12 ± 1·23)	(27·24)			
	3·00 ± 1·03	18·32	(10)	(11·45)	(n = 1L + 1M + 2S + 1S^v)
	(2·10 ± 1·00)	(18·34)			
	2·35 ± 1·10	14·35			
B. napus	(1·50 ± 0·93)	(13·10)			
	1·50 ± 0·09	9·16			
	(0·98 ± 1·03)	(8·55)			
	1·00 ± 0·49	6·00			
	(——)	(——)			

Data in parentheses are of samples from *B. napus*.

Table 5. Karyometrics of the populations a and b of *T. salignus*.

Name of the host plant	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
	4·10 ± 0·33	20·14		
	(2·90 ± 0·09)	(18·29)		
	3·50 ± 0·29	17·19		
	(2·75 ± 0·23)	(17·35)		
	3·30 ± 0·25	16·21		n = 1M + 5S + 1S^v
P. cassia	(2·50 ± 0·21)	(15·77)	20·35	
	2·80 ± 0·31	13·75		
	(2·35 ± 0·18)	(14·82)	(15·85)	
	2·55 ± 0·29	12·53		(n = 6S + 1S^v)
	(2·00 ± 0·13)	(12·61)		
	2·15 ± 0·27	10·56		
	(1·80 ± 0·17)	(11·35)		
	1·95 ± 0·30	9·58		
	(1·55 ± 0·18)	(9·77)		

Data in parentheses are of samples showing n = 6S + 1S^v.

Table 6. Karyometrics of the haploid set of chromosomes of *T. salignus* collected from *S. babylonica* showing n = 11.

Chromosome pair	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
I	5·00 ± 0·12	14·68		
II	4·25 ± 0·08	12·48		
III	4·00 ± 1·02	11·77		
IV	3·50 ± 0·08	10·27		
V	3·10 ± 1·23	9·10		
VI	3·00 ± 0·07	8·81	34·05	n = 4S + 7S^v
VII	3·00 ± 0·08	8·81		
VIII	2·50 ± 0·09	7·34		
IX	2·25 ± 0·08	6·60		
X	1·90 ± 0·07	5·58		
XI	1·55 ± 0·09	4·55		

could be readily identified as the diploid number and the relative length varies widely between them (table 7).

Cytological characterisation at the higher taxonomic categories of aphids is also noteworthy from our study. For example the genus *Aphis* of the tribe Aphidini is cytologically distinguished as 2n = 8 with n = 1L + 2M + 1S from that of the genus *Myzus* of the tribe Macrosiphini with 2n = 12; n = 2M + 2S + 2S^v. Similarly cinarine aphids feeding on Cupressaceae, have n = 6 as opposed to n = 5 for other *Cinara* feeding on Pinaceae (Blackman 1980; Das *et al* 1985).

It is apparent that karyotypic variation within the species seems to occur frequently in aphids, which have been recently reviewed elsewhere (Blackman 1980). Differences in diploid number (other than polyploidy) may be the resultants of dissociation or fusions involving the elements of normal set, or due to the presence of additional supernumerary or B-chromosomes. Exchange of chromosomal segments of unequal length results detectable structural heterozygosity in karyotype.

Table 7. Karyometries of the haploid set of chromosomes of *S. rosaeiformis* and *M. rosae* (in parenthesis).

Chromosome pair	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
I	3·58 ± 0·23	21·21		
	(2·49 ± 0·09)	(26·80)		
II	2·42 ± 0·22	14·34		
	(2·16 ± 0·08)	(23·38)		
III	2·15 ± 0·19	12·74		n = 1M + 3S + 5S^v
	(1·99 ± 0·08)	(21·38)	16·88	
IV	1·85 ± 0·18	10·96		
	(1·47 ± 0·07)	(15·80)	(9·30)	
V	1·60 ± 0·23	9·48		(n = 3M + 2S)
	(1·19 ± 0·07)	(12·72)		
VI	1·45 ± 0·19	8·59		
	(——)	(——)		
VII	1·38 ± 0·16	8·18		
	(——)	(——)		
VIII	1·25 ± 0·08	7·41		
	(——)	(——)		
IX	1·20 ± 0·07	7·11		
	(——)	(——)		

Chromosomal rearrangements are associated particularly with anholocyclic aphids, *M. persicae* and its asexual offshoots provide most studied example of this phenomenon (Blackman 1985).

In *M. persicae* polymorphism is very common in natural populations which are the products of autosomal dissociation giving 2n = 13 karyotype or autosome 1,3-translocation, resulting in 2n = 12 with a marked structural heterozygosity. Takada *et al* (1978) could rear a triploid clone (2n = 18) of *M. persicae* in the laboratory which originated by the fertilization of an unreduced diploid oocyte nucleus of autosome 1,3-translocation variety, by a normal haploid gamete. Morphologically it differed from the diploid form having a broader, more globose shape and thicker and shorter appendages and biologically it is less fecund. Such triploid clone if exists in nature and accordingly collected, might get a status of a new species unless the knowledge of its origin and cytology is known. In the field of agriculture, horticulture, forestry, misidentification of aphids may seriously affect the control strategies. Here we can cite the example of cytological separation of *Rubus* feeding *Amphorophora* species which were morphologically identified as one and the same species (Blackman *et al* 1977). Karyological investigation in taxonomic studies therefore seems logical.

In this connection it may be pointed out that DNA determination can be applied for further karyotype analysis since the amount of DNA/genome is usually constant and characteristic for each species, yet may differ considerably between species. If the karyotype is variable with large chromosome number, comparison of DNA content may provide clues to the nature of differences. If there are differences in chromosome number between the karyotype of different species, the DNA determination will show whether higher numbers are likely to be due to polyploidy. For example, Kuznetsova (1975) compared DNA content of *Anuraphis subterranea* (n = 13) with that of *A. farfarae* (n = 6). The larger n-value of *A. subterranea* was clearly not due to

polyploidy, as the DNA value for this species was less than that for *A. farfarae*. However, such a study has not yet been attempted by Indian cytologists and the same is true for gel electrophoretic studies as well.

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Recent advances in biosystematics of *Trichogramma* and *Trichogrammatoidea* (Hymenoptera, Trichogrammatidae)

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Abstract. Among the egg parasites used extensively for biological control of various lepidopterous pests all over the world the species of *Trichogramma* and *Trichogrammatoidea* (Hymenoptera, Trichogrammatidae) are the most important. The mode of reproduction of most of these is arrhenotoky while a few in *Trichogramma* is thelytoky, the later could be facultative with change in temperature, from one mode to the other. Since 1977, a large number of new species were described and studies on different aspects made world over. The studies made include crossing experiments which proved existence in nature of sibling species and other categories of incipient speciation which otherwise are difficult to discern. Studies on isozyme and enzyme analyses showed important differences among species and populations of *Trichogramma*, so also response by these to different chemical insecticides. Scanning electron microscopy is yet another important tool to supplement other differentiating factors. Other biological characters aiding systematics include sex ratio, life and fertility table studies showing breeding potential, host preferences, temperature tolerance, etc.

Keywords. Arrhenotoky; thilytoky; sibling species; isozyme; enzyme.

1. Introduction

Species of *Trichogramma* and *Trichogrammatoidea* (Hymenoptera, Trichogrammatidae) are amongst the most important egg parasites of mostly Lepidoptera, the latter mostly being noxious pests of agricultural and horticultural crops. Because of the comparative ease with which most of these egg parasites could be mass produced on laboratory factitious host insects, these are being released against target pests for biological control all over the world, especially in the People's Republic of China, USSR and USA.

Most species are arrhenotokous while a few in *Trichogramma*, e.g. *T. embryophagum* Htg. are thelytokous. However, there are instances of change in mode of reproduction with change in temperature.

This article contains information on the biosystematic studies made on these parasites after 1977 when a comprehensive article on this subject was published (Nagarkatti and Nagaraja 1977). As per our present understanding on the trend of organic evolution, the modern systematics should be as comprehensive as possible. The present emphasis on the subject takes into consideration not only the taxonomy based until recently on external morphology, but also genetics, ecology and other biological parameters. An attempt in this direction is made in this paper on the two genera of the family Trichogrammatidae.

Since 1977 (Nagarkatti and Nagaraja 1977), quite a number of papers on the complex components of biosystematics of these genera have appeared. These include crossing experiments, bioecology, biochemistry, effect of chemical pesticides and temperature tolerance in addition to descriptions of new species based mainly on

morphological characters. The recent literature on these is too complex and voluminous to condense in a short article like this. However, an attempt is made here to project some salient points on the subject.

Voegele and Pintureau (1982) based on available literature listed known 64 species of *Trichogramma* and divided them into 14 groups with description of two new species. They also included a key to differentiate these groups based on male genitalia and, in addition, made a detailed study on 10 species amongst them. Nagaraja (1978a) made a detailed biosystematic study on 10 species and a subspecies of *Trichogrammatoidea* using numerical taxonomy with 45 characters. Further to this, a detailed experimental hybridisation work was made (Nagaraja 1978b) and life and fertility tables on 6 species and a hybrid were worked out (Nagaraja 1986).

A large number of taxonomic papers describing new species all over the world have appeared in recent years. Existence in nature of sibling species, semispecies and other intraspecific categories are being recognised. This has enhanced the complexity of modern systematics and restricted the role of museum taxonomist specially of groups like Trichogrammatidae to identify a given taxa without understanding the supplementary information on biology, etc.

2. Crossing experiments

2.1 *Trichogramma*

This section deals with the discovery of 4 new species on the basis of morphology and results of crossing experiments made between these and other known related species. The new species are named tentatively as follows:

Trichogramma sp. A. — Ex *Agrius convolvuli* (L.) (Lep., Sphingidae) on *Colocasia antiquorum*, Vijayapura, 25 km away from Bangalore.

Trichogramma sp. B. — Ex *Danaus chrysippus* (L.) (Lep., Danaidae) on *Calotropis gigantea*, Saligrama (Dakshina Kannada Dist.), Bangalore.

Trichogramma sp. C. — Ex *Scirpophaga incertulas* (Walker) (Lep., Pyralidae) on *Oryza sativa*, Hebbal.

Trichogramma sp. D. — Ex *A. convolvuli* on *C. antiquorum*, Mandya.

The species A and C are closely related to each other and *T. hesperidis* Nagaraja and *T. flandersi* Nagaraja and Nagarkatti. Reciprocal crosses were arranged as shown below:

TA × TC	TB × TD	TD × *T. chilotraeae*
TA × *T. hesperidis*	TC × *T. hesperidis*	TD × *T. achaeae*
TA × *T. flandersi*	TC × *T. flandersi*	

Trichogramma sp. D (TD) resembles partly *T. chilotraeae* Nag. and Nagar. and *T. achaeae* Nag. and Nagar. The number of parental females used was 9–50 in one to 6 replicates. The F_1 progeny produced were from 7–956 with the female progeny produced from 0–21·24%. In normal breeding, each species produced 60–75% females. The total progeny produced by each parental female allowed with alien males was 0·5–31·22 per female while the average progeny from the homogamic

matings was above 60 per female. The males in these crosses usually were aggressive and less discriminatory with alien females but the females mostly were discriminatory against these males.

2.2 *Trichogrammatoidea*

Nagarkatti and Nagaraja (1977) mentioned the series of crossing experiments made on *Trichogramma* and *Trichogrammatoidea*. In the latter, 5 populations of *T. bactrae bactrae* Nag. when crossed between them showed considerable degree of reproductive isolation in some of them suggesting possibilities of incipient genetic divergence towards speciation.

Additional interesting experiments were made by the author in the Philippines on 3 populations of *T. cojuangcoi* Nag., the egg parasite of the cocoa moth, *Conopomorpha cramerella* (Snell.) (Lep., Gracillariidae) in Southeast Asia. These populations were collected from Mindanao (Philippines), Sabah (Eastern Malaysia) and Halmahera (Indonesia) islands attacking the same host (table 1).

T. robusta is a distinct species, differing morphologically from *T. cojuangcoi*. There is extremely restricted gene exchange with *T. cojuangcoi*. The hybrids from all these crosses did not survive for raising hybrid cultures. The stocks from the islands of Mindanao, Negros and Luzon (all Philippines) were interfertile. However, the ones from Mindanao and Negros produced lesser F_1 progeny.

Table 1. Crossing experiments between 3 populations of *Trichogrammatoidea cojuangcoi* from Mindanao (Philippines), Sabah (E. Malaysia) and Halmahera (Indonesia), *T. robusta* and two of the above populations and 3 stocks (including Mindanao's) of *T. cojuangcoi* within the Philippines.

Pairs-crossed ♂♂ ♀♀	Parental ♀♀ used	No. Repl.	F_1 Progeny ♂♂	♀♀	♀♀(%)	Per parental ♀
Phil × Halm	125	5	2,601	245	8·61	22·77
Halm × Phil	90	4	1,591	86	5·13	18·63
Sabah × Halm	100	4	1,328	19	1·41	13·47
Halm × Sabah	75	3	1,127	33	2·85	15·47
Phil × Sabah	105	5	2,030	157	7·18	20·83
Sabah × Phil	105	5	1,758	233	11·70	18·96
Cavt × Mind	21	1	336	339	50·22	32·14
Mind × Cavt	25	1	374	485	56·46	34·36
Mind × Bacd	50	2	383	177	31·61	11·20
Bacd × Mind	50	2	413	321	34·86	12·68
T.rb × Mind	75	3	1,198	12	1·07	16·13
Mind × T.rb	75	3	2,120	12	0·56	28·43
T.rb × Sabah	50	2	1,037	31	2·90	21·36
Sabah × T.rb	75	3	2,300	30	1·29	31·07

Phil, Philippines; Halm, Halmahera; Sabah, Sabah populations of *T. cojuangcoi*; Cavt, Cavite; Mind, Mindanao; Bacd, Bacolod stocks of *T. cojuangcoi*; *T. rb*, *T. robusta*.

3. Biological observations

3.1 *Scanning electron microscopy*

The earliest study of *Trichogramma* using scanning electron microscopy was made by Voegele *et al* (1975) who studied female antennae and found differences in the number of sensillae on ventral part of the club in 3 species studied, *T. maltbyi* Nag. and Nagar., *T. evanescens* Westwood and *T. brasiliensis* (Ashm.). Preliminary attempts made by Dr S Nagarkatti in USA (Anon 1972) on male genitalia *in situ* and male antennae of *Trichogramma* sp. confirmed the relative position of different genitalic parts and sensillae respectively, that were observed earlier under microscope. Pointel (1977) also made a similar study in France. Hung (1982) reported existence of petal like structures on distal end of tibia of both sexes of 6 species of *Trichogramma*. These were thin and transparent, used probably for transferring secretion from abdomen to the wings to increase aerodynamic function (Hung 1982) and possibly diagnostic. Wang *et al* (1986) observed two ring segments in antennae in both sexes of *T. dendrolimi* Mats., and in other species as well, one free and another firmly attached to the base of funicle. Both these were observed earlier by the author under microscope, but since one is indistinct, it has been shown in all drawings in a dotted line.

3.2 *Behaviour in parasitism*

Thorpe and Dively (1985) observed in the laboratory the parasitism of *Heliothis virescens* (F.) (Lep., Noctuidae) eggs in 1·8 l (13 × 13 cm), 43 l (23 × 23 × 62 cm) and 400 l (2 × 2 × 1 m) arenas with soyabean foliage as the substrate, by *Trichogramma minutum* Riley, *T. pretiosum* Riley and *T. exiguum* Pinto and Platner. The trend of parasitism by each species, especially *T. minutum* and *T. pretiosum*, differed.

Intraspecific differences towards certain hosts have been observed in France by Ferreira and Pintureau (1983) in *T. minutum*. A strain of this species was reported to be the best in controlling *Mythimna unipuncta* (How.) (Lep., Noctuidae). Glas *et al* (1981) found that *T. cacoeciae* Marchall laid slightly more eggs in *Mamestra brassicae* (L.) (Lep., Noctuidae) and rejected *Pieris brassicae* (L.) (Lep., Pieridae) from a distance even without antennating them while *T. evanescens* laid more eggs in *P. brassicae*.

The biological differences between two sibling species, *Trichogrammatoidea lutea* Gir. and *T. prabhakeri* Nag. were shown (Nagaraja 1986). These differences include (i) sex ratio, 1♂ : 1·131 ♀♀, and 1♂ : 4·324 ♀♀; (ii) the finite rate of natural increase (λ), 1·4677 ♀♀/♀/day and 1·4223 ♀♀/♀/day; (iii) generation time (T) 10·0309 and 9·7608 days and (iv) net female production (R_0) 46·910 and 31·147 ♀♀/♀/generation, respectively.

3.3 *Role of temperature*

The role of temperature in biological differences between populations and species of *Trichogramma* has to be emphasised. Voegele and Russo (1981) observed in France that in *T. rhenana* Voegele and Russo, parasite of *Ostrinia nubilalis* (Hub.) (Lep., Pyralidae) the threshold temperature for development was 15°C and the species was

able to develop at the maximum of 34°C while *T. schuberti* Voegele and Russo was found to develop at temperature as low as 11°C but did not survive above 33°C. Russo and Voegele (1982) working on 4 species, the above two and *T. nubilale* Ertle and Davis and *T. maidis* Pintureau and Voegele at temperatures ranging from 11°C to 34°C observed that there were two groups each having distinct temperature requirement.

Cabello and Vargas (1985) found that *T. cardubensis* Vargas and Cabello being thelytokous at 20°C for 41 generations changed to arrhenotoky above 28°C and reverted to thelytoky at 20°C. Whether this is also a case of thelytoky to amphitoky and vice versa has to be further investigated.

3.4 *Studies on isozymes and enzymes*

A large number of papers have been published in recent years on this subject on *Trichogramma.* Hung (1982) compared electrophoretically collected samples of *T. pretiosum* from Maryland, Arkansas, Georgia and Texas (USA) and found differences in the isozyme analyses of different enzymes and discovered a sibling species on the basis of these studies. Enzymatic study of 10 strains from 9 species of *Trichogramma* were made by Pintureau and Babault (1982). The esterases, malate-dehydrogenases and tetrazolium-oxidases allowed distinguishing of related species, supplementary information on them and distant species. The groupings based on this study conformed to the ones made on the basis of external morphology. As a result of work on *T. evanescens, T. chilonis* Ishii, *T. pretiosum, T. cacoeciae, T. pieris* and *T. dendrolimi,* Cao *et al* (1986) suggested use of esterase isozyme analyses that could aid taxonomy of the genus. Hung and Huo (1985) studied the electrophoretic banding patterns for the malic enzyme, phosphoglucomutase and phosphoglucose isomerase in 20 laboratory cultures of 14 species of *Trichogramma.* Variations in PGM in *T. exiguum, T. marylandense* Thorpe and *T. pretiosum,* in PGI in *T. exiguum, T. marylandense, T. minutum* and *T. perkinsi* Gir., while in ME only in *T. pretiosum* were observed by these authors.

Hung (1982) reported that the studies made on caryotypes of *T. chilonis, T. evanescens, T. nubilale* Ertle and Davis and *T. pretiosum* did not show significant differences and all had n = 2 sm + 2T + 1 A. However the species could readily be distinguished by zymograms of superoxide dimutase and esterase.

3.5 *Response to insecticides*

Chemical insecticides have been tested on *Trichogramma* in several countries. Kapustina (1977) found that Trichlorphon and Phosalone at 0·01% would not affect different races of *T. evanescens* and *T. cacoeciae pallida* Meir of Orshan race, *T. embryophagum* Hertig from Byelorussia but suggested releases not earlier than 3 to 4 days after sprayings. In India Paul (1986) mentioned that toxicity level of a particular insecticide possibly differed on different species of *Trichogramma* while synthetic pyrethroids, fenvalerate and flucythrinate, monocrotophos, endrin, phosalone and endosulfan were relatively safer to *Trichogramma.* The species studied were *T. chilonis, T. brasiliensis, T. japonicum* (Asm.) and *T. perkinsi.* The nomenclature of the last species needs confirmation.

4. Discussion

The importance of biosystematics has been stressed in dealing especially with sibling species, semispecies and biological races which are abound in *Trichogramma* and *Trichogrammatoidea*. It is the realistic approach to understand the complex ramifications of the process of evolution. The taxonomists of the above mentioned genera till fifties of this century were attempting to classify them on the basis of colour, length of antennal hairs, wing fringe, etc. Attempts were made to study male genitalia in *Trichogramma evanescens* by Hintzelman (1925) and other species by Ishii (1941) and Tseng (1965). It was in 1968 that Nagarkatti and Nagaraja made a detailed study of male genitalia of *Trichogramma* by interpreting and naming various parts of the genitalia. Subsequently the authors discovered a large number of new species from India and elsewhere. Taking cue from this work which laid down certain criteria as diagnostic to the taxonomy of the above two genera, entomologists working on them world over discovered numerous new species. According to the latest list compiled by Voegele and Pintureau (1982), Pang (unpublished results) and some more new species yet to be described by the author, there are over 85 species of *Trichogramma* and 25 of *Trichogrammatoidea* known to date as compared with 19 and 8 species, respectively, prior to 1968 (*Trichogramma*) and 1978 (*Trichogrammatoidea*).

Apart from the taxonomic studies based on male genitalia and other external characters, a large series of crossing experiments conducted during the past two decades brought to light existence of sibling species, semi-species, etc and those of disputed status requiring further investigations. Thus, two stocks of *T. minutum*, one on either side of Rocky Mountains (USA), did not interbreed, showing reproductive isolation (Nagarkatti and Fazaluddin 1973). Such species need to be considered as sibling species. The other examples include *Trichogramma* sp. A, *T.* sp. B and *T. hesperidis* Nag., mentioned in the text. Few more examples are *T. japonicum* and *T. pallidiventris* Nag. in *Trichogramma* and *T. lutea* Gir. and *T. prabhakeri* Nag. and the 3 distinct populations of *T. cojuangcoi* in *Trichogrammatoidea*. In addition, there are numerous morphologically indistinguishable species, but spatially widely separated whose status has to be determined by using biological methods. To quote an example *Trichogrammatoidea guamensis* Nag. is described from material collected in Guam on eggs of *Lampides boeticus* (L.) (Lep., Lycaenidae) and *T. cojuangcoi* in Southeast Asia. These two parasites are almost indistinguishable morphologically from each other. But owing to nonavailability of live material from Guam, the crossing trials could not be made. However, none of the several hundreds of eggs on *L. boeticus* on *Crotolaria* sp. and *Cajanus cajan* collected in India and the Philippines and Papua New Guinea, was parasitised by *T. guamensis*. The only parasites reared from these eggs were an unidentified species of *Telenomus* (Hym., Scelionidae) and *Trichogrammatoidea armigera* Nag., *T. guamensis* and *T. cojuangcoi* could possibly be sibling species. However, until results of crossing experiments on these parasites are available, their taxonomic status remains provisional.

In the section on crossing experiments, one could see that *T.* sp. A, *T.* sp. C and *T. hesperidis* produced extremely few progenies per parental female (0·50–4·00/ parental female). This happened possibly due to the mechanical injury in the female reproductive system during mating by alien males (heterogamic matings) or antigenic reaction in the female to the alien sperms, indicating mechanical or chemical

isolation, common in heterogamic matings. This being apart, there are instances like restricted progeny production even with a substantial number of female production as observed in the cross between Bacolod (Negros) and Mindanao populations of *T. cojuangcoi*. This could be the positive instance of incipient divergence on way to speciation.

The problem with sibling species where the reproductive isolation has not resulted in external manifestation has to be fully understood. This phenomenon is very frequent in these parasites. This factor therefore has to be borne in mind by the taxonomist when identifying such species from spatially isolated areas. Another factor worth mentioning is that the sympatric diverging populations of a species show greater reproductive isolation than the allopatric ones, thus perpetuating the evolutionary process and preventing wastage of sperms. This is possibly the instance of character displacement (Brown and Wilson 1956) observed in ants. An almost similar instance was observed in *Trichogramma* (Nagaraja 1973).

The data on biological parameters should also be taken into consideration like the ones occurring in *T. lutea* and *T. prabhakeri* (Nagaraja 1978b). These should include host and habitat preferences in the intraspecific populations, within the ambit of species. *T. lutea* and *T. prabhakeri* and Halmaheran, Sabah and Philippine populations of *T. cojuangcoi* could be considered as semispecies with respect to each other although there appeared to be greater reproductive isolation between the former two species which ultimately may develop into full species. There are hence innumerable intraspecific forms allover the world, each having attained different levels of distinctiveness in the process. The other examples in parasitic Hymenoptera include *Aphytis* spp. (Hym., Aphelinidae) (Rao and DeBach 1969a,b).

The study on isozymes of enzymes is very important step in the modern approach to systematics. Where the morphological distinctiveness is lacking, these analyses and the reaction of each parasite species or population to a given chemical pesticide add to the systematics on the group of the said parasite. The numerical taxonomy as explained by Sokal and Sneath (1963) is the most logical expression of biosystematics with respect to the groups like *Trichogramma* and *Trichogrammatoidea* with all their cryptic species and other incomplete evolutionary stages.

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Biosystematic studies on *Aphytis* in India—A promising area of research

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Abstract. Species of the genus *Aphytis* Howard (Hymenoptera, Chalcidoidea, Aphelinidae) develop as primary ectoparasites of armoured scale insects (Homoptera, Coccoidea and Diaspididae) and are the most important natural enemies of this group of pests. Several species have been successfully employed in the biological control of important armoured scale insect pests in different parts of the world. In spite of the importance of *Aphytis*, very little is known about the species occurring in India, a promising area for additional and important species. In the past although taxonomists have made significant contributions to our knowledge of Indian Chalcidoidea, the genus *Aphytis* has largely been neglected because the identification of the species in this group is extremely difficult for want of readily recognizable morphological criteria and due to the common occurrence of sibling and uniparental species. The recognition of sibling species and different biological races is often important in biological control. Hence there is a need in India to initiate studies on *Aphytis* to establish the identity of cryptic species and to recognize biological races and other infra-specific entities exhibiting morphological and biological differences, with special attention to those parasitic on armoured scale insects on economically important plants. These studies would pave the way for biological control strategies for pest species in India and elsewhere and also for those which may eventually attain pest status due to changing ecosystems.

Keywords. *Aphytis*; diaspine scale insects; sibling species.

1. Introduction

Armoured scale insects (Homoptera, Coccoidea and Diaspididae) have attained great importance particularly as pests of fruit trees and ornamentals, owing to high reproductive capacity, survivability and difficulty in controlling them with insecticides. Species of the genus *Aphytis* Howard (Hymenoptera, Chalcidoidea, Aphelinidae) are primary ectoparasites of these insects. The adult *Aphytis* female pierces the armour with the ovipositor and lays one or several eggs on the body of the host. Well protected by the armour, the larvae feed on the body fluids of the host and develop, pupating below the empty armour. In addition to the scale insects killed by parasitism, considerable mortality is caused due to the predatory host-feeding by the adult *Aphytis* females. *Aphytis* spp. are well recognised as superior in effectiveness to the endoparasites and predators of armoured scale insects (Rosen and DeBach 1979). Several species have been successfully employed in biocontrol projects and several others could be instrumental in keeping the populations of many potential pests at subeconomic levels.

Identification and separation of the species of *Aphytis* are extremely difficult, due to the minute size and relative scarcity of reliable distinguishing characters. The diagnostic adult morphological characters of value for the identification of *Aphytis* species include (i) number of antennal segments; (ii) ratio of length to width of the antennal club; (iii) ratio of the lengths of the various antennal segments;

(iv) number, size and relative melanization of the setae on the mesonotal sclerites; (v) shape of the crenulae on the posterior margin of the propodeum; (vi) number of setae in the abdominal cerci; (vii) wing shape; (viii) ratio of the length of the longest seta in the marginal fringe of the forewing to the greatest width of the disk; (ix) number of setae along the marginal vein; (x) number of bullae along the submarginal vein; (xi) number of setae in the delta area of the forewing; (xii) relative visibility of the interommatidial setae on the compound eye; (xiii) sculpture of the thorax; (xiv) general colour of live adults; and (xv) degree of melanization and its location. However there are considerable intraspecific variations in the length and number of setae and wing proportions but proportions of the antennal segments are quite reliable. In certain cases the relative length of the ovipositor to the size of the specimen may aid in specific identification. Though variable in number with the size of the specimens, the propodeal crenulae, particularly the shape which is quite stable, are useful. The degree of coarseness and melanization of the body setae and the wing pattern are quite stable. The pattern of pigmentation of the body and appendages is the least variable character. A combination of several characters, studied under binocular stereoscopic microscope and high-power phase-contrast microscope help in separating certain species from the others. Examination under a scanning electron microscope has helped to identify minute diagnostic morphological differences, thus resolving the status of some sibling species.

Extensive research on *Aphytis* by DeBach and his co-workers and others (cited in Rosen and DeBach 1979) has revealed that this group consists of rapidly evolving and highly specialised insects exhibiting different degrees of morphological and biological distinctness and the common occurrence of sibling and uniparental species, thus necessitating biosystematic studies. It is now well recognised that virtually no species of *Aphytis* can be identified for certain on morphological characters alone. In practical biological control, not only the recognition of cryptic species but even biological races is of great importance.

2. Biological tools in systematics of *Aphytis*

While a brief account of biological attributes with reference to their utility in systematics of *Aphytis* is given here, no attempt is made to discuss biosystematics of *Aphytis* which has been reviewed by Rosen and DeBach (1979).

2.1 *Morphology of eggs and larvae*

While the eggs of *Aphytis* spp. are indistinguishable, slight differences in shape of mandibles, spiracles, spiracular tubercles, number and position of minute cuticular tubercles and pores in the cephalic area of the third instar larvae are observed. Although some of these characters differ in the representatives of different groups, they are of little practical value.

2.2 *Pupal pigmentation*

It is an important supplementary diagnostic character that proved to be the first clue to indicate the distinctness of some cryptic species (e.g. *A. lingnanensis* Compere and

A. chrysomphali (Mercet)). Species that are almost inseparable in adult stage, e.g. *A. holoxanthus* DeBach, *A. melinus* DeBach and *A. fisheri* DeBach have distinct pupal pigmentation.

2.3 *Meconium*

A detailed study of the meconial pellets in 12 species, using scanning electron microscope, revealed specific differences in their size, shape and surface pattern which are characters useful in identification.

2.4 *Duration of life cycle*

Although it was observed that the duration of life cycle was longer in members of the more primitive species groups, the ranges overlapped in closely related species.

2.5 *Longevity and fecundity*

Longevity under various constant temperatures differed in species of *Aphytis*. However, it appears to be a difficult tool in practical systematics, in view of the necessity to conduct the study under strict standardised conditions. Although fecundity is characteristic of species, it is of some value only when the study is conducted under uniform conditions.

2.6 *Host range*

In well established cases of monophagy, the host record can be a short cut to the identification as with *A. lepidosaphes* Compere or when a species is known to restrict to a particular genus of the host, e.g. *A. cylindratus* Compere to *Pseudaonidia* spp.

2.7 *Cytological characters*

Preliminary studies on the number, shape and size of chromosomes of *Aphytis* have not yielded any useful criterion.

2.8 *Reproductive isolation*

The final proof of the specific status of bisexual organisms is reproductive isolation. Hence experimental hybridization is the most reliable tool in identification of sibling species. Extensive studies on experimental hybridization have revealed common occurrence of semispecies and sibling species in *Aphytis*, e.g. experimental hybridization between sibling forms, *A. lingnanensis*, *A.* '2002' and *A.* 'R-65-23', reared from different hosts and from different geographical areas exhibited that *A. lingnanensis* and *A.* 'R-65-23' were good species in relation to each other but both were semispecies in relation to '2002'. Methods to quantify the extent of reproductive and sexual isolation and criteria to delimit species and semispecies categories have been worked out, based on the coefficients of joint isolation.

2.9 *Thelytoky*

Although a different mode of reproduction is an indication of the existence of biological differences in *Aphytis*, there is occurrence of uniparental and biparental sibling forms with or without complete reproductive isolation. A further complication is the occurrence of uniparental sibling species with distinct biological attributes. For the purpose of utilization in biological control, it is necessary to recognise these, whatever their specific status may be.

2.10 *Ethological characters*

Direct observation of acceptance or rejection is a simple means of distinguishing live cultures and differences in mating behaviour appears to be consistent in different species and warrant further study.

2.11 *Biochemical characters*

Only preliminary studies using paper chromatography and slab-gel electrophoresis have been conducted which indicate a promising area of research.

3. Indian species of *Aphytis*

In their treatise on *Aphytis*, Rosen and DeBach (1979) listed 8 species from India. These records of *Aphytis* were a result of collections made in India for biocontrol in other parts of the world. These are listed below along with their hosts recorded in India:

Parasitoid	Host
A. coheni DeBach	*Aonidiella orientalis* (Newstead)
A. lepidosaphes Compere	*Cornuaspis beckii* (Newman)
A. lingnanensis Compere	*Aonidiella aurantii* (Maskell)
A. melinus DeBach	*A. aurantii*
A. paramaculicornis Rosen and DeBach	*Parlatoria oleae* (Colvee)
A. peculiaris (Girault)	*A. ?orientalis*
A. philippinensis DeBach and Rosen	*A. aurantii*
A. theae (Cameron)	*Fiorinia theae* Green

It is interesting to note that all the hosts listed above are major pests elsewhere but are of little importance in India. The *Aphytis* spp. listed against them are recognised as of great importance in biological control.

In the past although taxonomists have made significant contributions to our knowledge of Indian Chalcidoidea, work on *Aphytis* in India remained neglected until a survey was conducted by CIBC to record natural enemies of diaspine scale insects (Anon 1986). This yielded an additional 3 identified species and over 20 partly identified species and also many additional host records of species already known to

occur in India. The additional species are *A. holoxanthus* DeBach and *A. pinnaspidis* Rosen and DeBach from *Pinnaspis strachani* (Cooley), and *A. sankarani* Rosen and DeBach from *Pseudaulacaspis cockerelli* (Cooley) and *Aonidiella aurantii*, the last one being new to science and described along with another new species from India, *A. bangalorensis* Rosen and DeBach from *Pseudaulacaspis barberi* (Green) (Rosen and DeBach 1986).

4. Scope for future work

The partly identified species of *Aphytis* are only an indication of the scope for future work. The identity of many of these could be confirmed by biosystematic studies.

In India, diaspine scale insects of major economic importance include *Melanaspis glomerata* (Green) and *Quadraspidiotus perniciosus* (Comstock) and to-date there is no published record of indigenous *Aphytis* from these. Surveys for *Aphytis* parasitising these pests particularly on alternate hosts, might yield species which could be utilized in biological control. In India, *Aspidiotus destructor* Signoret is a sporadic pest on coconut and *Aonidiella aurantii*, though not a major pest on citrus, is important on rose. There is a need to study the role of *Aphytis* spp. as regulating factors of these scale insects on different plants and over wide areas. Biosystematic studies on *Aphytis* would have to be the foundation on which such studies could be based. Several diaspine scale insects occur in India which are serious pests elsewhere, e.g. *Chrysomphalus aonidum* (Linnaeus), *Parlatoria* spp., *Pinnaspis strachani* and *Cornuaspis beckii*. There is a need to learn about the *Aphytis* spp. parasitic on them which would prove helpful if these scale insects eventually attain pest status due to changing ecosystems. *Pseudaulacaspis pentagona* (Targioni-Tozzetti) occurs in India, and one of its hosts elsewhere is mulberry. In view of its potential pest status, it should also be included for surveys. Rosen and DeBach (1979) estimate a rough ratio of 1:1 between armoured scale insects and *Aphytis*. Varshney (1985) listed nearly 200 species of diaspididae from India. Therefore about 200 species of *Aphytis* could be expected from India. Since faunistic knowledge of this group in India, an area noted as one of the promising ones, is very restricted at present, there is an enormous scope for work on this group. To begin with, studies on *Aphytis* parasitic on those diaspididae which are pests and potential pests in India could be made. These would pave the way for biological control of diaspine scale insect pests in India and elsewhere. Special effort to study *Aphytis* reared from economically important diaspidid hosts on wild plants could prove rewarding rather than restricting to cultivated habitats which are subjected to heavy pesticide treatments, possibly resulting in complete exclusion of parasitoids.

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Feeding and behavioural parameters and egg ultrastructure in the biosystematics of Reduviidae (Insecta—Heteroptera)

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Abstract. The feeding behaviour exhibited by the different subfamilies of Reduviidae is distinctly correlated with the specific prey types involved. Important adaptive modifications are seen associated with their methods of prey capture and feeding. Based on the analyses of feeding behaviour, reduviids may be grouped as (i) 'blood feeding' type, (ii) 'sticky-trap' type, (iii) 'raptorial' type, (iv) 'wait and grab' type, (v) 'pin and jab' type and (vi) 'chase and pounce' type. The oviposition methods are highly varied in Reduviidae and it is possible to assign group characteristics, but the ultrastructure of the eggs, particularly those of chorionic collar extensions and the operculum, indicate subfamily resemblance. The usefulness of feeding and reproductive behaviours and the egg ultrastructure in the biosystematics of Reduviidae are discussed.

Keywords. Feeding and reproductive behaviours; egg ultrastructure; biosystematics; Reduviidae.

1. Introduction

Family Reduviidae comprises of a large group of terrestrial predatory bugs well represented in the Indian sub-continent as well as in the Continents of Africa and the Americas. The subfamily composition and the relationship of Reduviidae with other heteropterous families remain highly controversial till this date. China and Miller (1959) recognised 29 subfamilies under Reduviidae. The Phymatidae and Pachynomidae, considered as distinct families, have been relegated to subfamily level (Carayon *et al* 1958; Wygodzinsky 1944). Usinger and Wygodzinsky (1964) have shown that Phimophorinae and Mendanocorinae are very closely related and that both should be placed under one, thereby eliminating subfamily Mendanocorinae. The subfamily status of Visayanocorinae (Villiers 1948), Eupheninae, Tegeinae and Rhaphidosomatinae (Carayon *et al* 1958) are not acceptable to many. Davis (1969) considered subfamilies Apiomerinae, Diaspidinae, Ectinoderinae, Tegeinae, Harpactorinae and Rhaphidosomatinae as sharing several apomorphic characteristics and placed all these as Tribes under the subfamily Harpactorinae. He considered Phonolibinae and Tegeinae as not sufficiently different from each other to warrant separate group placements, and placed them under one tribe Tegeini. Inspite of all these changes, family Reduviidae still contains the highest number of subfamilies among all Heteropteran families. There is an absolute need for a complete reassessment of atleast the subfamily composition of Reduviidae. A comprehensive information from diverse sources like external organs, internal organs including gonads, male and female accessory glands, salivary glands, digestive organs, etc, chromosomal number, feeding and reproductive behaviours, and the egg structure would be required to make a true evaluation of the relationships between the taxa of this family. The present attempt tries to explore the usefulness of the feeding and reproductive behaviours and the egg ultrastructure in the biosystematics of Reduviidae.

2. Feeding behaviour

Reduviids occupy diverse habitats and feed on a variety of arthropods, exhibiting preferences for certain prey types. While Triatominae have specialised for vertebrate blood, Ectrichodiinae feed exclusively on millipedes, Piratinae prefer carabid beetles and gryllids, and the majority of Harpactorinae species attack soft-bodied prey like caterpillars, grubs and beetle larvae. While Rhaphidosomatinae feed on worker termites, Reduviine species predate on ants and bees. Inspite of the vast number, relatively nothing is known about the prey and feeding behaviour of a large number of reduviids. Their feeding behaviour consists of stimuli-response mediated sequences of events, always initiated by a moving prey (Haridass and Ananthakrishnan 1980a) and varies according to the prey types involved. It is possible to construct distinct feeding behavioural models for the different species and based on the present studies and on those reported elsewhere, the reduviid feeding behaviours can be categorised into 6 major types.

2.1 *'Sticky-trap' type*

Species of Apiomerinae and Ectinoderinae exhibit this special type of feeding behaviour (Miller 1971). Members of these subfamilies are known to deliberately smear on their fore-legs slow-drying resins from trees like *Pinus*, *Acacia* and *Agathis* and keeping such resin coated legs extended in front of them they wait for small flying insects to get entangled and trapped which are ultimately fed. In many of these species the fore-legs are never used for locomotion (table 1).

2.2 *'Raptorial' type*

Species exhibiting this type of behaviour are endowed with long fore-legs, armed with spines, tubercles and setae. These predators lie in wait on vegetation and floral parts or at suitable places for their prey, consisting mostly of Psocids, Culicids and Drosophilids. With quick, flicking movement of the raptorial fore arms the prey is caught and killed. Phymatinae, Physoderinae, Bactrodinae and Emesinae exhibit this kind of feeding behaviour (Miller 1971; Wygodzinsky 1966) (table 1).

2.3 *'Wait and grab' type*

Species showing this kind of feeding behaviour are more active than types 1 and 2. They move quickly and are provided with powerful forelegs, possessing well-developed tibial pads. They predate on medium-sized prey like Termite workers, bugs, bees and ants and sulk near places where their prey types frequent like termitaria, ant-hills and bee-hives. On sighting a prey they quickly approach and grab it with their forelegs, select suitable site for insertion of stylets and injection of toxic saliva and kill the prey. Though they are good hunters, active chasing of the prey is not exhibited by these predators belonging to the subfamilies Stenopodinae, Salyavatinae and Reduviinae (Ambrose *et al* 1986a,b; Haridass 1985a; Livingstone and Ambrose 1978a,b; Miller 1971; Odhiambo 1958a) (table 1).

Table 1. Feeding behaviour in Reduviidae.

Feeding type	Subfamily	Prey type	Rostrum/degree of forward movement	Fore legs. Tibial pads. Gait.
Blood feeding	Triatominae	Vertebrate blood (cattle, dog, rat, man)	Long, slender, straight. $\geqslant 180°$	Thin, long. Reduced or absent. Slow.
Sticky trap	Apiomerinae Ectinoderinae	Small flying insect (small bees)	Short, slender, curved. $\geqslant 45°$	Thin, long, setose. Absent. Slow.
Raptorial	Phymatinae Physoderinae Emesinae Bactrodinae	Small flying insects (Psocids, Culicids Drosophilids)	Short, slender, curved. $\geqslant 45°$	Thin, long, spinose, raptorial. Absent. Slow.
Wait and grab	Stenopodinae Salyavatinae Reduviinae	Medium and moderate sized insects (ants, bees, bugs, termites)	Short, stout, curved. $\geqslant 45°$	Short, stout. Well-developed. Fast.
Pin and jab	Rhaphidosomatinae Harpactorinae	Soft, bodied, slow moving insects (caterpillars, grubs, beetle larvae)	Long. slender, curved. $\geqslant 90°$	Thin, long. Reduced or absent. Sluggish
Chase and pounce	Piratinae	Robust, fast-moving insects beetles, gryllids Grasshoppers	Short, stout, curved. $\geqslant 45°$	Short, very stout. Very well-developed.
	Ectrichodiinae	Millipedes		Very fast.

2.4 *'Pin and jab' type*

Species of both Harpactorinae and Rhaphidosomatinae exhibit this kind of feeding behaviour. Their prey types are usually soft-bodied and slow moving ones, like caterpillars, grubs and beetle larvae. On sighting, these predators approach the prey slowly and with the long rostrum jab at places behind the head and inject toxic saliva. They follow the violently wriggling prey without taking the stylets out of the prey body. When the size is big, they pin down the prey with the fore legs, either before and or after salivary injection. When small, the prey is completely lifted off the ground and held dangling at the rostral tip till complete immobilisation occurs. The prey is held in this position till the entire body contents are sucked out. Slow gait is very characteristic of these predators and active chasing of the prey is not a component of their feeding behaviour. Also in these reduviids, feeding sites are not often changed as is the case with types 3 and 5 predators (Edwards 1962, 1966; Haridass 1985a; Miller 1971; Parker 1969, 1971, 1972) (table 1).

2.5 *'Chase and pounce' type*

As a rule the predators exhibiting this kind of feeding behaviour (Piratinae and Ectrichodiinae) are robust and active runners, with short powerful legs. On sighting a moving prey, which themselves are active forms (Carabid beetles, Gryllids and Millipedes), the predators quickly run and pounce on the prey, grabbing and holding with tibial pads of the fore and mid legs. This is followed by quick immobilisation by injecting toxic saliva after suitable site selection in the intersegmental membranes at the bases of the antennae, or neck or thoracic regions or as in the case of millipedes (Ectrichodiinae) always at the bases of legs near the cephalic region. After killing the prey, the predators spend considerable time in cleaning their body of the defense secretions emitted by the prey during predation and this behaviour is an important component of their feeding behaviour. Once cleaning is completed, the predators again insert the stylets, drag or pull the dead prey to a secluded place and feed. Feeding sites are changed frequently as and when one part of the prey is emptied (Ambrose *et al* 1985a, b; Haridass 1985a; Haridass and Ananthakrishnan 1980a, b; Livingstone and Ambrose 1984; Miller 1953, 1971) (table 1).

2.6 *'Blood feeding' type*

Species of Triatominae are specialized for haematophagy and unlike in all other types, the temperature gradient emanating from the vertebrate host like cattle, dog, rat and man, initiates the feeding responses of a hungry insect. After orientation towards the host and its contact with the antennal tip, the long rostrum is extended forwards and used to probe and select a suitable site after sampling of the blood meal is accomplished. Feeding continues from the same site till the insect is satiated, without disturbing the host. Unlike other reduviids, the triatomine bugs are capable of surviving long periods of starvation (Haridass and Ananthakrishnan 1981; Lent and Wygodzinsky 1979) (table 1).

3. Adaptive modifications

Many structural features are found correlated with the feeding behaviour of reduviids and these are effectively used during prey capture and feeding, and they are of great survival value to these predators.

3.1 *Tibial pads*

The tibial pads are characteristic features of many reduviids and are found at the tip of the forelegs and in some species also in the mid legs. The tibial pads are pin cushion-like structures with numerous tenet hairs supported by a layer of secretory epithelial cells at the base. These cells produce an oily secretion that flow to the tips of these hairs (Edwards 1962; Gillett and Wigglesworth 1932; Miller 1938, 1942; Wigglesworth 1938). The shape and size of these tibial pads vary in different species (Miller 1971; Livingstone and Ambrose 1984) and the tenet hairs also differ in different species (Haridass and Ananthakrishnan 1980b). The hairs at the tibial pads, with an oily substance smeared on them, serve to increase the static tension, thereby enhance the gripping ability during prey capture. The tibial pads exhibit their maximum development in the 'chase and pounce' type; they are equally or moderately developed in the 'wait and grab' type, reduced or absent in the 'pin and jab' type and totally wanting in the 'raptorial' and 'sticky trap' types. In the 'blood feeding' type, they are poorly developed, though gripping of the prey is never involved.

3.2 *Rostrum*

The length of the rostrum and the extent to which it can be flexed and moved forwards are also correlated with the feeding behaviours of the reduviids. The rostrum is short and curved, capable of little forward movements in the 'sticky trap' and 'raptorial' types. During prey immobilisation and feeding the rostrum is moved forward only to about 45°. In the 'wait and grab' as well as in the 'chase and pounce' types, the short, curved, but stout rostrum is capable only of limited forward movement to about 45°. In the 'pin and grab' type, the rostrum plays an important role in prey capture. It is long, slender and capable of being projected forward to about 90°. These rostrum enable the predators to probe and select suitable sites on the prey body and to insert stylets from a distance. They are also helpful to lift smaller prey from the ground, thus avoiding the chance of prey escape. Maximum forward projection of the rostrum is met with the species of Triatominae, where the rostrum is capable of forward movement to about 180°. These blood feeders probe and select suitable sites on the host body from a safe distance without disturbing the vertebrates (Haridass and Ananthakrishnan 1981; Lent and Wygodzinsky 1979).

3.3 *Camouflage*

Nymphal camouflage is common among some species of Reduviinae, Stenopodinae, Salyavatinae (Livingstone and Ambrose 1978b; Odhiambo 1958b; Miller 1953, 1971)

and Triatominae (Zeledon *et al* 1973). The nymphs of these insects accumulate and deliberately cover their body with dead remains of the prey, soil particles and debris. Sticky secretions from special hairs on their body are used for this purpose. Such camouflaging behaviour is not only of great adaptive value to escape from the cannibalistic behaviour of other nymphs, but also to escape detection from other predators.

4. Reproductive behaviour

4.1 *Copulation*

Two distinct types of copulatory behaviours are common in Reduviidae. Members of Piratinae alone remain in copula in an end-to-end posture after the males successfully engage their copulatory organs with the females. Interestingly their claspers are asymmetrical, the right being smaller than the left. Species belonging to all other subfamilies of Reduviidae assume a lateral copulatory posture, the males after successfully engaging the aedeagus, remain on the lateral aspects of the females. In many species exhibiting such a posture, pre-copulatory ride by the males on the females for long periods is very common.

4.2 *Oviposition*

The methods of depositing eggs and the oviposition behaviours differ considerably among Reduviidae. Broadly, egg deposition may be categorised into (i) exposed type and (ii) concealed type, though variations do occur in each of these types. Eggs may be loosely laid, scattered and exposed on the soil, under stones and boulders or in crevices as in Reduviinae, Salyavatinae and Triatominae. The eggs may be buried individually with their apices alone exposed as in Piratinae and Stenopodinae. The eggs may be laid attached to suitable substratum at the ground level or on vegetation as in Ectrichodiinae, Harpactorinae, Rhaphidosomatinae and Emesinae. Eggs are deposited either as fewer eggs per batch in numerous clutches as most of the type I do, or eggs may be deposited in large numbers per batch in fewer clutches as in type II. Reduviids that scatter the eggs do not show any sequence in the arrangement of eggs on the soil. Those that deposit eggs in crevices also do not exhibit any order in the placement of the eggs. Piratine species that bury their eggs select moist, shady places and using the ovipositors make holes in the soil and deposit individual eggs exposing the anterior part outside. The females also use their hind legs to cover the exposed part of the eggs with sand and soil particles. Reduviids that deposit masses of eggs attached to suitable substratum, particularly Harpactorinae, exhibit regular sequence in attaching these with cementing materials from their accessory glands. Instances of parental brooding of eggs are reported only from this subfamily.

4.3 *Egg*

A true operculum covering the mouth of the egg like a lid of the jar, well-defined sealing bar, enabling the operculum to fit into the egg, exo- and endochorionic

layers, well-developed aero-micropylar system placed around the collar and a continuous inner aerostatic layer are characteristic features of reduviid eggs which enable their identification from the rest of the eggs of the heteropterous families (Cobben 1968; Haridass 1985b, 1986a,b; Hinton 1981; Readio 1926; Southwood 1956).

Since the chorion is the product of the ovarian epithelial cells, its surface architecture is more or less uniform throughout the family, though variations do exist in terms of the number and size of the chorionic follicular cells, and the pits, warts and tubercles contained within these cells, as well as the thickness of the cell boundaries. But the characteristics offered by the chorionic collar region and the operculum are of considerable value as taxonomic tools in recognising various subfamilies of Reduviidae:

Eggs of reduviids exhibiting exposed oviposition, freely scattering the eggs in the soil, or depositing them inside open crevices (Reduviinae, Salyavatinae and Triatominae) are characterised by simple chorionic architecture, very simple collar rim as well as simple operculum. But the eggs of each of these subfamilies offer enough characters to distinguish one from the other. The ovoidal eggs of Triatominae are the simplest with thin, convex, disc-like operculum, the surface of which is marked by polygonal cells (figure 1B). The collar rim is without any extensions and it imperceptibly merges with the body of the egg (Haridass 1986b). The spherical eggs of Salyavatinae are easily distinguished by the triangular shape of the operculum (figure 1D) containing short, porous extensions restricted to a central disc-like area. Eggs of Stenopodinae are ovoid-spheres and possess short, highly porous collar extensions. Their operculum also shows spongy, vertical projections distributed throughout the upper surface (figure 1F). Reduviinae eggs are spherical possessing short collar extensions. Their operculum also shows warty projections throughout (figures 1G and 2B). The oval eggs of Ectrichodiinae and Tribelocephatinae superficially resemble the eggs of Triatominae in having simple chorionic markings and in the absence of collar extensions; but the operculum possess spongy respiratory structures, particularly predominant in the periphery (figure 1C) (Haridass 1986b).

As against the shape of the eggs of reduviids with exposed and scattered oviposition habits, the shape of reduviid eggs that are glued to the substratum, either individually as in Emesinae, or in fewer numbers as in Rhaphidosomatinae or as large masses as in Harpactorinae, is elongated, cylindrical or bottle-shaped. The chorionic collar of Emesinae is simple while that of Rhaphidosomatinae is short and highly porous (figure 1E). While the opercular surfaces of the former contain variously modified outgrowths, that of the latter always possess a central conical disc-like elevation (figure 2F). The chorionic surface of Harpactorine eggs exhibits deep pits and follicle cells and the eggs of this subfamily also show greatest development (figure 2A) and modification of both the collar rim and opercular extensions. Invariably the collar extensions and those of the operculum (figure 2E) are united (Haridass 1986a). Eggs of Piratinae, like those of Harpactorinae, are easily distinguishable. These ovoidal eggs possess a conical projection at the posterior pole containing numerous pore canals opening into the inner aerostatic layer (figure 2D). The chorionic collar gives out numerous, highly porous extensions in the form of long filamentous extensions (figure 1A). Their operculum is distinctly disc-shaped (figure 2C) with porous extensions, highly variable as those of Harpactorine eggs (Haridass 1985b).

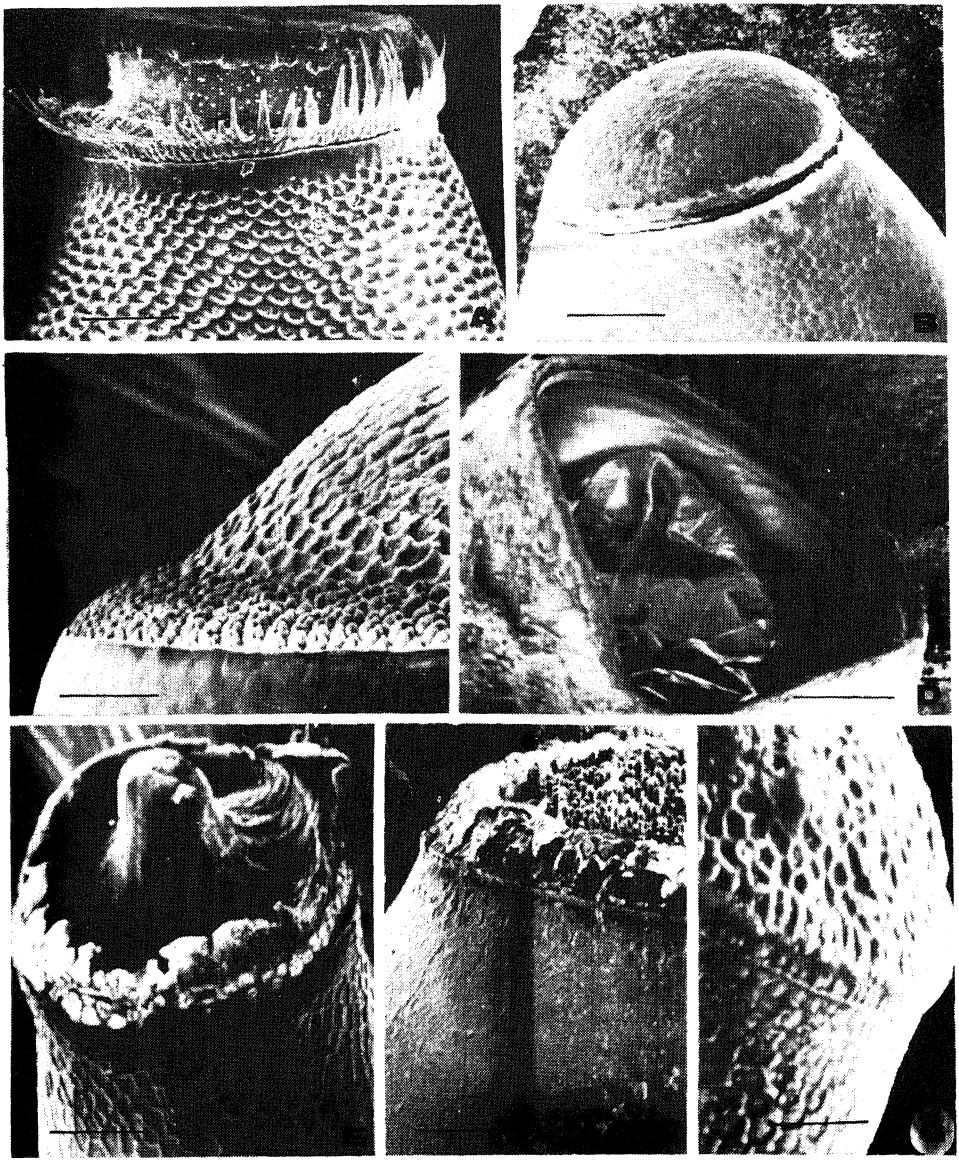

Figure 1. Eggs of Reduviidae. **A.** *Ectomocoris tibialis.* Anterior half of egg with operculum (scale, 66 μ). **B.** *Linshcosteus costalis.* Anterior half of egg with operculum (scale, 64 μ). **C.** *Haematorrhophus nigroviolaceus.* Anterior part of egg with operculum (scale, 64 μ). **D.** *Lizarda annulosa.* Anterior half of egg without operculum (scale, 87 μ). **E.** *Rhaphidosoma atkinsoni.* Anterior half of egg with operculum (scale, 66 μ). **F.** *Oncocephalus annulipes.* Anterior part of egg with operculum (scale, 73 μ). **G.** *Acanthaspis pedestris.* Anterior part of egg with operculum (scale, 64 μ).

5. Discussion

Feeding behaviour in Reduviidae involves a series of stimuli-response sequences of events and these behaviours exhibited by different species are distinctly correlated

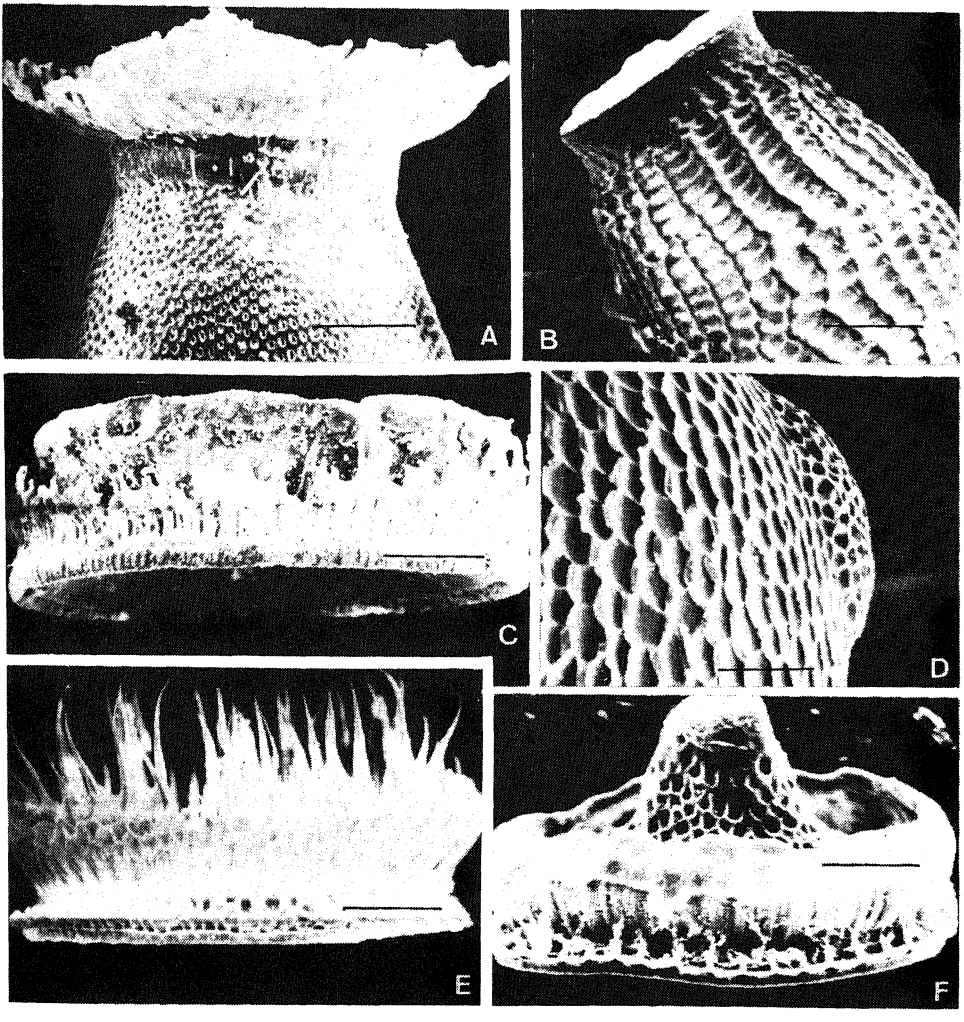

Figure 2. Eggs of Reduviidae. **A.** *Rhinocoris fuscipes.* Anterior half of egg with operculum (scale, 84 μ). **B.** *Acanthaspis* sp. Anterior half of egg with operculum (scale, 89 μ). **C.** *Ectomocoris tibialis.* Operculum (scale, 78 μ). **D.** *Pirates affinis.* Posterior part of egg (scale, 74 μ). **E.** *Rhinocoris fuscipes.* Operculum (scale, 92 μ). **F.** *Rhaphidosoma atkinsoni.* Operculum (scale, 76 μ).

with the prey types involved. There is definitely preference for specific prey types in many sub-families and based on the analyses of their behaviour it is possible to construct feeding behaviour models applicable to different subfamilies (Haridass and Ananthakrishnan 1980a). While the temperature gradient emanating from the vertebrate host is the initiating factor for feeding responses in the haematophagic Triatominae (Haridass and Ananthakrishnan 1981), moving prey triggers the chain of events in all other reduviids (Haridass 1986a; Haridass and Ananthakrishnan 1980a; Livingstone and Ambrose 1978a,b; Parker 1969, 1971, 1972). The feeding behaviour of Triatominae stands out as a very special and distinct type not met with other Reduviidae. The 'sticky trap' type of feeding exhibited by subfamilies

Apiomerinae and Ectinoderinae, using resins or gums of *Acacia*, *Pinus* and *Agathis* trees on the fore legs to capture small flying insects like sticky flypaper (Miller 1971), may also be considered as special type of feeding behaviour not met with other reduviid taxa. The next complex type of feeding behaviour is that of the 'raptorial' type, shown by the members of Phymatinae, Physoderinae, Emesinae and Bactrodinae (Miller 1953, 1971; Wygodzinsky 1966). These predators lie in wait at strategic places and capture small insects that approach them with their raptorial arms. In all these 3 categories, the reduviids effectively and efficiently use the variously modified fore legs for prey capture. The total absence of tibial pads is very striking in these forms. Also in these the rostrum is short, thin and curved and capable of only limited forward movement, but sufficient to kill and suck the prey body contents.

Both the 'wait and grab' as well as the 'chase and pounce' reduviids depend very much on their fore legs for prey capture and the highly developed tibial pads enhance the gripping ability of the predators making it almost impossible for the prey to escape the hold once caught. The former type of predators frequent the abodes of their prey and quickly grab and kill without the necessity to chase the prey. While the latter type reduviids are good runners and efficiently chase and capture the prey, though many a times the prey capture is violent and the predators are subjected to rough treatments (Haridass and Ananthakrishnan 1980a). In both these predators the rostrum is short and curved but very stout, capable of limited forward movements. Predators of the 'pin and jab' type are slow movers possessing long, thin legs with or without tibial pads, enabling them to follow the slow moving soft-bodied prey with a few steps. With the long slender proboscis, capable of more than 90° forward extension, they reach, probe and kill the prey from a safe distance; the fore legs are frequently used to restrict the movement and escape of the prey.

No generalisations can be drawn from the copulatory behaviour of reduviids. The common types of copulatory postures, the end to end and the lateral, are also common in numerous other families of Heteroptera.

Oviposition in Reduviidae occurs in diverse habitats and inspite of the variety, eggs are found in the same situation as one finds the males and the females themselves. Again, no generalisation can be made in Reduviidae on the basis of the modes of oviposition. But when one takes into consideration other characters like shape of the egg, structure of chorionic collar rim and operculum, there is an indication of subfamily resemblence in the eggs. The triatomine eggs appear to be the simplest of all the eggs in having simple operculum and collar rim. Eggs of Salyavatinae are unique in having triangular operculum shown by no other reduviid subfamily, though the significance of this is not clear. Piratine eggs are also unique in possessing the conical projection at the posterior pole with pore canals, the filamentous collar extensions, and the highly porous operculum. All these are respiratory structures transporting ambient oxygen to the developing embryo and the structural adaptations as a whole are related to the ovipositional habits of Piratinae where individual eggs are buried vertically into the soil with apical parts alone exposed (Haridass 1985b). Eggs of Harpactorinae are also quite distinct, not only in being cylindrical with rounded posterior pole, but also in possessing most complex chorionic collar and opercular extensions, the collar extensions forming a continuous sheet and frequently united with the opercular extensions. Though the eggs' shape of Emesinae and Rhaphidosomatinae are similar, both these can be easily differentiated on the basis of the collar extensions and the characters of the operculum. In collar rim

Table 2. Reproductive behaviour and eggs of Reduviidae.

Subfamily	Copulatory posture	Oviposition	Egg		
			Shape	Chorionic collar extensions	Operculum
Triatominae	Lateral	Concealed loosely scattered/ crevices	Oval		Simple, convex-disc; no out-growth
Emesinae	„	Exposed-individually attached/vegetation	Cylindrical		Disc-like; short vertical, scattered outgrowth
Salyavatinae	„	Exposed-loosely scattered/ soil	Spherical Triangular Operculum	Simple extensions absent	Convex-disc; short blunt vertical, porous; scattered
Reduviinae	„	Concealed-loosely scattered/ crevices	Oval/ spherical		Convex-disc; short warty, porous outgrowths; scattered
Tribelocephalinae	„	Exposed-laid as loose mass/ ground	Oval		Convex-disc; short, warty, porous outgrowth restricted to periphery
Ectrichodiinae	„	Exposed/buried as loose mass/ground	Oval		
Stenopodinae	„	Partially exposed individually/soil	Ovoid spherical	Short, vetical continuous	Button-like, short blunt vertical, porous outgrowth scattered
Rhaphidosomatinae	„	Exposed individually attached/vegetation	Cylindrical	porous	Disc-like with central conical outgrowth
Harpactorinae	„	Exposed-attached in mass/ vegetation	Bottle-shaped	Long, vertical continuous net-work	Button-like; long vertical outgrowth united with collar extensions
Piratinae	End to end	Partially exposed individually buried/soil	Oval conical posterior pole	Long, porous filamentous	Button-like; oblique filamentous outgrowth

characters the eggs of Stenopodinae are similar to those of Rhaphidosomatinae, the former can be easily differentiated by the ovoid-spherical shape and in the possession of short porous opercular extensions. Eggs of the subfamilies Reduviinae, Tribelocephalinae and Ectrichodiinae share common features in the simple collar rim region; again the opercular characteristics offer scope for differentiating these 3. Tribelocephaline eggs resemble the Ectrichodiinae eggs even in the opercular characters and it is interesting to note that these two subfamilies also share many internal characters (Louis and Kumar 1973) and these are also considered as related (Davis 1961, 1966).

As has been shown in tables 1 and 2 it is possible to group the different subfamilies of Reduviidae into certain categories based on the feeding and reproductive behaviours and on the basis of egg ultrastructure, though such grouping need not reflect their true relationship. Based on such limited studies pertaining to a few of the subfamilies firm conclusions on the inter-relationships between the subfamilies of this large family cannot be arrived at. But such studies based on behavioural parameters and stable internal characters and egg structure are of considerable assistance in establishing relationships (Louis and Kumar 1973). In view of the large size of the family and its subfamily composition, analyses of the feeding and reproductive behaviours as well as the egg structure of other sub families are required to arrive at logical and acceptable evaluation of the interrelationships among Reduviidae.

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Biological, behavioural and morphological tools in the biosystematics of Reduviidae (Insecta—Heteroptera—Reduviidae)

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Abstract. Biosystematic studies of 3 subfamilies of Reduviidae viz Acanthaspidinae, Harpactorinae and Piratinae have provided sufficient data for clear demarcations of these subfamilies in terms of biological, behavioural and morphological tools. Biological considerations involve incubation and stadial periods, hatchability, adult longevity, sex ratio and morphometric analyses of life stages. Behavioural parameters include nature of predators, camouflaging, death feigning, head nodding, spitting, nymphal rolling, mating and oviposition of the members of these 3 subfamilies. Information on the bioecology of 25 species, behavioural aspects of 40 species and morphological aspect of 165 species serve to adequately augment our understanding of the biosystematics of this important predatory group of insects.

Keywords. Biosystematics; Reduviidae; Acanthaspidinae; Harpactorinae; biological; behavioural Piratinae, morphological tools.

1. Introduction

Members of Reduviidae constitute one of the major groups of predatory insects and many of them are reported to be predating on a number of insect pests of agriculture and forestry and efforts have been made to employ them as agents of biological control against insect pests (Ambrose 1987). Distant (1903, 1910) in his Fauna of British India volumes described and classified 342 species of Reduviids under 106 genera and 13 subfamilies based on morphological characters. Samuel and Joseph (1953), Muraleedharan (1976) and Ambrose and Livingstone (1986a, b) described two species of *Rhinocoris*, two species of *Henricohahnia*; species of *Rhinocoris* and a new genus namely *Neohaematorrhophus* (*N. therasii*) respectively. Goel (1984) reported the taxonomic relationships of the subfamilies of Reduviidae as indicated by the thoracic skeleton. Ambrose (1980, 1987) and Livingstone and Ambrose (1978a, b, 1984) discussed the biological, behavioural and morphological peculiarities of subfamilies of Reduviidae. Haridass (1985, 1986a, b) described the ultrastructure of the eggs of 4 subfamilies of Reduviidae. No information is available on the biological, behavioural and morphological parameters in the biosystematics of Reduviidae. Informations on the bioecology of 25 species, behavioural aspects of 40 species and morphological aspects of 165 species augment our understanding of the biosystematics of this important predatory group of insects. The present paper deals with the biological, behavioural and morphological tools derived from 85 species which can be used to clearly demarcate 3 subfamilies of Reduviidae viz Acanthaspidinae, Harpactorinae and Piratinae.

2. Materials and methods

The species studied presently are given in table 1. Insects were collected from the scrub jungles, semiarid zones and tropical rain forest of Southern India. They were

Table 1. Species of Reduviids studied.

I. ACANTHASPIDINAE

1. *Acanthaspis bistillata* Stål x, 2. *A. pedestris* Stål xxx, 3. *A. siva* Dist xxx, 4. *A. livingstonei* sp. nov., 5. *A. philomanmariae* sp. nov. xxx, 6. *A. subrufa* Dist. x, 7. *A. quinquespinosa* Fabr. xxx, 8. *A. zebraica* Dist. x, 9–17. *Acanthaspis* spp. (9), 18. *Edocla slateri* Dist. xxx, 19. *E. pelia* Dist. x, 20. *Linshcosteus cornifex* Dist. x, 21. *Linshcosteus* sp., 22. *Pasira perpusilla* Walk. x, 23. *Sminthocoris fuscipennis* Stål x, 24. *Triatoma rubrofasciatus* De geer x, 25. *Velitra sinensis* Walk., 26. *Velitra* sp.

II. HARPACTORINAE

27. *Coranus atricapillus* Dist. x, 28. *C. spiniscutis* Reut. x, 29. *C. vitellinus* Dist. xxx, 30–31. *Coranus* spp. x (21), 32. *Cydnocoris gilvus* Burm. x, 33. *C. crocatus* Stål x, 34. *Endochus cingalensis* Stål x, 35. *E. inoratus* Stål x, 36. *E. migratorius* Dist. x, 37. *Euagoras plagiatus* Burm. xxx, 38. *E. typicus* Dist., 39. *Irantha armipes* Stål xx, 40. *I. consobrina* Dist. x, 41. *Lophocephala querini* Lap. xxx, 42. *Polididus armatissimus* Dist. xxx, 43. *Rhaphidosoma atkinsoni* Bergr. xx, 44. *Rhaphidosoma* sp., 45. *Rhinocoris fuscipes* Fabr. xxx, 46. *R. kumarii* Amb. and Liv. xxx, 47. *R. longifrons* Stål xxx, 48. *R. marginatus* Fabr. xxx, 49. *R. marginellus* Fabr. xxx, 50–55. *Rhinocoris* spp. (6), 56. *Sphadanolestes aterrimus* Dist. xxx, 57–58. *Sphedanolestes* spp. (2) x, 59. *Sycanus ater* Wolff x, 60. *S. reclinatus* Dohra xxx, 61. *S. pyrrhomelas* Walk. xxx, 62–63. *Sycanus* spp (2) x.

III. PIRATINAE

64. *Catamiarus brevipennis* Serv. xxx, 65. *Ectomocoris atrox* Stål x, 66. *E. cordatus* Wolff x, 67. *E. cordiger* Stål x, 68. *E. elegans* Fabr. x, 69. *E. erebus* Dist. x, 70. *E. gangeticus* Bergr. x, 71. *E. ochropterus* Stål, 72. *E. quadriguttatus* Fabr. x, 73. *E. tibialis* Dist. xxx, 74. *E. vishnu* Dist. xxx, 75. *Ectomocoris* sp. xxx, 76–78. *Ectomocoris* spp. (3) x, 79. *Pirates affinis* Serv. xx, 80. *P. quadrinotatus* Fabr. x, 81–82. *Pirates* spp. (2), 83. *Sirthenea flavipes* Stål x.

x—Morphological characters studied; xx—Behavioural and morphological characters studied; and xxx—Biological, behavioural and morphological characters studied.

References: Ambrose (1978, 1980, 1983, 1986), Ambrose and Livingstone (1978, 1979, 1984, 1985a, b, 1986a, b, c, d), Ambrose *et al* (1985), Livingstone and Ambrose (1978a, b and 1984) and Vennison and Ambrose (1986).

reared in the plastic containers (12 × 6 × 4 cm) under laboratory conditions. (temperature −32°C; RH 80–85% and photoperiod 12 to 13 h) on houseflies, grasshoppers, catterpillars and camponotine ants. Eggs laid by the insects were collected and allowed to hatch, and the nymphs hatched were reared in the plastic containers. Observations like incubation period, stadial period, hatchability, adult longevity and sex-ratio were made. Morphometric analyses of life stages were carried out under the microscope with micrometers. Behaviours like predation, nymphal camouflaging, death feigning, head nodding, spitting, nymphal rolling, mating and oviposition were studied both in the field and in the laboratory. For morphological studies, structures like egg, leg, rostrum, tibial pad and wings were treated with 10% KOH, 5% glacial acetic acid, dehydrated in alcohol, cleared in zylol, mounted in canadabalsom or DPX and observed under the microscope. Indices like oviposition days index (percentage of egg laying days in the total adult female longevity), tibial index (area of tibial pad ÷ area of tibia), lateral oviduct index (length of lateral oviduct ÷ length of ovary) and egg index (length of egg ÷ length of adult female insect) were calculated.

3. Observations

The biological, behavioural and morphological tools derived from 85 species belonging to 24 genera to demarcate subfamilies Acanthaspidinae, Harpactorinae and Piratinae are presented in tables 2–7.

3.1 *Biological tools*

Tables 2 and 3 show the biological demarcations among these 3 subfamilies as indicated by the incubation and stadial periods, time of eclosian and ecdysis, adult longevity, sex ratio, preoviposition period, fecundity rate and hatchability.

3.2 *Behavioural tools*

3.2a *Predatory behaviour*: Table 4 exhibits the predatory behavioural tools to demarcate the 3 subfamilies as indicated by their acts (figures 1–3), the duration of acts, different grades of nymphal congregational feeding and cannibalism (figure 7).

3.2b *Mating behaviour*: Mating behavioural demarcation of these subfamilies are clearly seen in the acts of mating, duration of mating acts, premating and postmating periods, ejection of spesmatophore capsule, frequency of mating and the interval between two successive matings (table 5 and figures 4–6).

3.2c *Oviposition behaviour*: Eggs are laid above or just below the soil surface in Acanthaspidinae, above the leaves or trunk of plants or on stones in Harpactorinae and below the soil surface in Piratinae. Members of Acanthaspidine and Piratinae

Table 2. Biological tools to demarcate the subfamilies of Acanthaspidinae (A) Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Incubation period (days)			
	18 ± 5·5	11·1 ± 3·1	27·3 ± 10·7
	(9–45)	(6–23)	(13–46)
Eclosion	Early morning	Afternoon	Night
Stadial period (days)			
Shortest	II (or) III	II (or) III	
	(II = III)	(II III)	II
I–Adult	91 ± 19·9	62·8 ± 20	120·8 ± 54
	(63–117)	(41–105)	(75–215)
Ecdysis	Night (or) early morning	Afternoon	Night
Adult longevity (days)			
Male: Female	90:110	50:50	43:62
	(50–201:47–204)	(13–72:14–80)	(28–70:43–96)
Sex ratio			
Male: Female	1:0·9	0·6:1·0	1·0:0·8
Generation	Multi voltine	Multi voltine	Univoltine

Numbers in parentheses indicate the range ($\bar{x} \pm SD$)

Table 3. Biological tools to demarcate the subfamilies Acanthaspidinae (A) Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Female adult longevity in days	113 ± 56 (52–185)	59·4 ± 37·2 (21–138)	52·5 ± 4·5 (48–57)
Preoviposition period in days	39 ± 11 (26–59)	21·1 ± 10·5 (8–42)	14 ± 1 (13–15)
Total no. of batches of eggs	31·6 ± 13·3 (14–55)	9·6 ± 5·1 (2–15)	17 ± 2 (15–19)
Total no. of eggs	121 ± 37 (62–166)	180·2 ± 72·6 (15–279)	93·5 ± 19·5 (74–113)
Average no. of eggs/batch	4 ± 1 (2–5)	23·1 ± 20 (3–68)	6 ± 2 (4–8)
Minimum no. of eggs/batch	1 ± 0	11·2 ± 17·79 (1–58)	2 ± 0
Maximum no. of eggs/batch	9 ± 1·5 (7–11)	81·9 ± 30·9 (5–97)	10 ± 1 (9–11)
No. of nymphs hatched	49 ± 22 (20–87)	61·5 ± 40 (12–144)	20 ± 6 (14–26)
Hatching (%)	48·2 ± 21 (28–85)	78·8 ± 12·11 (64–100)	25·5 ± 2·5 (23–28)
Frequency of hatching (0%)	14 ± 13 (1–28)	2·4 ± 1·4 (0–4)	9 ± 1 (8–10)
Frequency of hatching (100%)	7 ± 5 (2–15)	4·8 ± 2·3 (1–7)	1·5 ± 0·5 (1–2)

Numbers in parentheses indicate the range ($\bar{X} \pm SD$).

Table 4. Predatory behavioural tools to demarcate the subfamilies Acanthaspidinae (A), Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Acts of predation			
Arousal	Fast (1–4 s)	Slow (5·6 s)	Instantaneous
Approach	Fast (1–20 s)	Slow (or) waiting (20 s 1 h)	Aggressive (1–4 s)
Capturing	Pouncing with legs (3–30 s)	Pinning with rostrum (10 min–3·5 h)	Pouncing with legs (2–15 s)
Paralysing	Slowly (10 s–2 min)	Quickly (2–60 s)	Slowly (20 s–3 min)
Sucking	Firm predator grip released but hold the prey with legs (21 min–3 h)	Lifting the prey with the rostrum (or) holding the prey with legs (20 min–1·5 h)	Predator grip released and sucked at its ease (1 h–6 h)
No. of sucking sites	More (12–20)	Less (8–15)	More (20–30)
Nymphal congregational feeding	In early instars	In all instars	Absent
Nymphal cannibalism	In all instars	In early instars	In all instars

Values in parentheses indicate the range.

Table 5. Mating behavioural tools to demarcate the subfamilies Acanthaspidinae (A), Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Premating period in days	Short (9–15)	Long (10–20)	Shortest (6–8)
Post mating period prior to oviposition	Long (12–39)	Shortest (5–20)	Longest (31–40)
Acts of mating	Fast	Slow	Fastest
Arousal	(2 s–20 min)	(2 s–20 min)	(2–3 s)
Approach	Similar to Predatory (3–40 s)	Lethargic (10 s–10 min)	Aggressive (1–5 s)
Precopulatory cannibalism of incompatible partner	Rarely present	Absent	Present
Precopulatory riding	Absent	Present (8 s–3 h)	Absent
Duration of copulation	Short (9 min–2 h)	Long (20 min–8 h)	Very short (17–20 min)
Position of copulation	Side to side	Side to side and V end to end	End to end and V side to side
Time for ejection of spermatophore capsule	More (34 min–8 h)	Less (7 min–4 h)	More (40 min–8 h)
Post copulatory cannibalism	Present	Rarely present	Predominantly present
Frequency of mating	Highest (8–34)	Intermediate (10–20)	Lowest (6–8)
Interval between mating	Short (1 h–7 days)	Long (1–36 days)	Long (4–19 days)

Values in parentheses indicate the range.

Table 6. Morphological tools to demarcate the subfamilies Acanthaspidinae (A), Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Colour			
Adults	Warning < dull	Dull < bright	Warning
Nymphs	Brown	Ochraceous	Brownish black < black
Wing	Sexual dimorphism male-alate female-apterous (or) micropterous	Alate	Sexual dimorphism male-alate female-apterous and alary polymorphism in both sexes
Rostrum	Curved	Straight (or) slightly curved	Deeply curved
Nymphal hairs	Straight hairs and stiff tufts of hairs at the dorsum and margin of abdomen	Straight and club shaped hair all over the body	Only straight hairs
Egg	globose > oval	Elongate	Elongately oval
Tibial pad	Well developed	Absent > poorly developed	Highly developed

Table 7. Morphometric analytical tools to demarcate the subfamilies of Acanthaspidinae (A), Harpactorinae (H) and Piratinae (P).

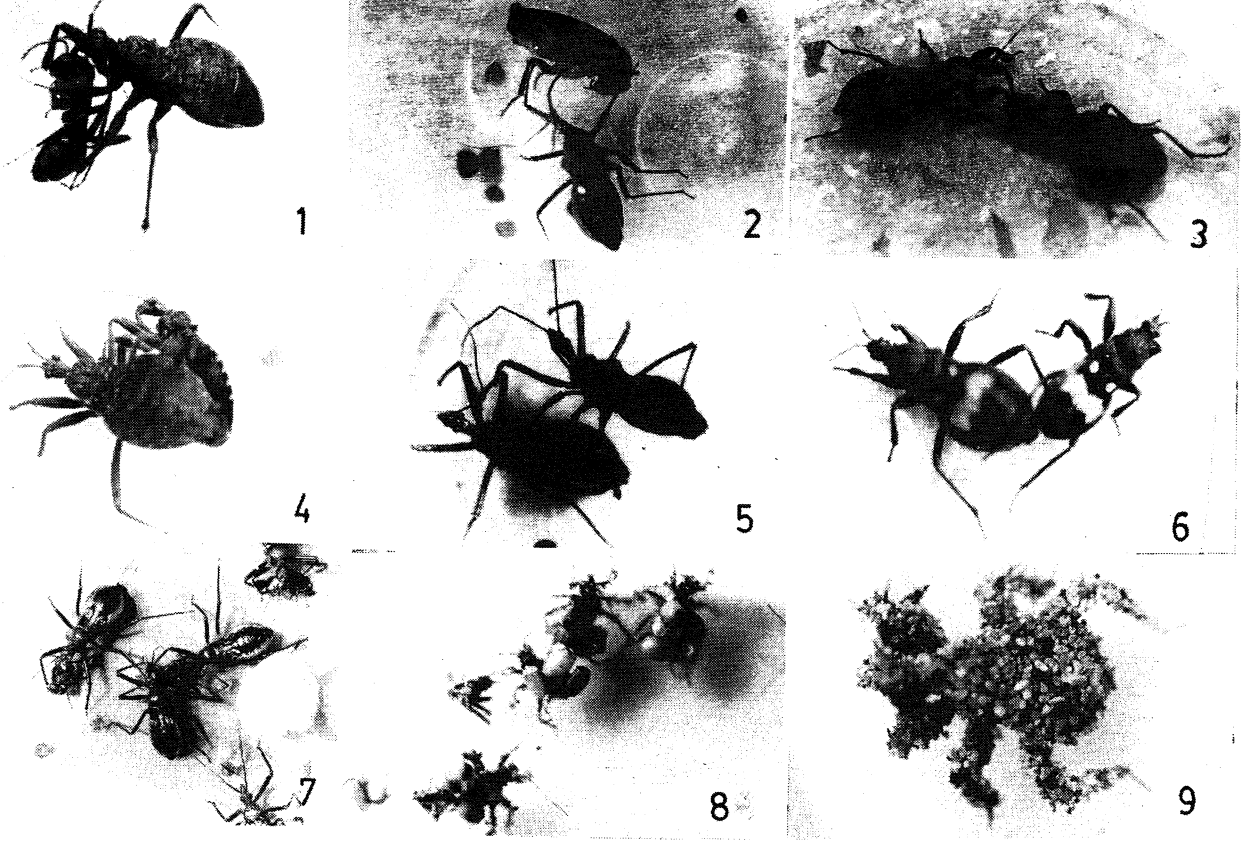
Tools	A	H	P
Anteocular postocular length	AO = PO (50%) or AO < PO (50%)	AO < PO (60%) AO = PO (40%)	AO > PO (100%)
Antennal segments			
Shortest	Scape	Pedicel	Scape
Longest	I Flagellar	Scape (40%) (or) II Flagellar	Flagellar
Rostral segments			
Shortest	Medial	Terminal (60%) Basal (40%)	Medial
Longest	Basal (50%) (or) Terminal (50%)	Medial	Basal (50%) (or) Terminal (50%)
Prothorax length : width	1 : 08	1 : 09	1 : 07
Tibia shortest	Fore (or) Mid	Mid	Mid
Fore tibial index	0·1 ± 0·04	0·05 ± 0·02	0·05 ± 0·03
$\left(\frac{\text{Area of fore tibia}}{\text{Area of insect}}\right)$	(0·03–0·15)	(0·02–0·14)	(0·01–0·12)
Fore tibial pad index	0·23 ± 0·15	0·04 ± 0·02	0·63 ± 0·26
$\left(\frac{\text{Area of fore tibial pad}}{\text{Area of fore tibia}}\right)$	(0·12–0·54)	(0·01–0·06)	(0·2–1·02)
Egg index	0·07 ± 0·01	0·12 ± 0·04	0·1 ± 0·01
$\left(\frac{\text{Length of egg}}{\text{Length of insect}}\right)$	(0·05–0·09)	(0·06–0·18)	(0·09–0·11)
Lateral oviduct index			
$\left(\frac{\text{Lateral oviduct length}}{\text{Ovary length}}\right)$	0·11 ± 0·03	0·1 ± 0·02	1·6

lay the eggs loosely but the former prefer to glue the eggs to fresh excreta. Harpactorinae reduviids lay the eggs in definite pattern and the eggs are cemented with each other and to the substratum (table 8).

3.2d *Nymphal camouflaging*: Present only in Acanthaspidinae (figures 8 and 9).

3.2e *Death feigning*: Observed only in Harpactorinae.

3.2f *Head nodding*: Obscurely developed in Harpactorinae, present in Acanthaspidinae and aggressively developed in Piratinae with 'crich-crich' sound as a defensive mechanism.



Figures 1–9. **1.** *A. pedestris* exhibit Acanthaspidine type predation. **2.** *R. marginatus* show characteristic Harpactorine prey lifting. **3.** *P. affinis* displays piratine predation at ease. **4.** *A. pedestris* at side to side position of mating. **5.** *R. kumarii* at precopulatory riding over. **6.** *C. brevipennis* at side to side copula. **7.** *R. marginatus*-nymphal congregational feeding. **8 and 9.** *A. pedestris*-nymphal camouflaging.

Table 8. Ovipositional, nymphal camouflaging, death feigning, head nodding and spitting behavioural tools to demarcate the subfamilies Acanthaspidinae (A), Harpactorinae (H) and Piratinae (P).

Tools	A	H	P
Oviposition			
Eggs laid	Joint below (or) above the surface of soil	On tree trunks, leaves and stones	Deep inside the soil
Mode of egg laying	Loosely, preference to glue the eggs to fresh excreta	In batches eggs are cemented together and to the substratum	Loosely no preference to glue the eggs to fresh excreta
Nymphal camouflaging	Present	Absent	Absent
Death feigning	Absent	Present	Absent
Head nodding	Present	Obscurely present	Aggressive with crich-crich sound
Spitting of watery saliva	Absent	Present	Absent

3.2g *Spitting of watery saliva*: Only the members of Harpactorinae spit the watery saliva at provocation.

3.3 *Morphological tools*

Morphological tools like colour of adults and nymphs, nature of wing, rostrum, nymphal hairs, eggs and tibial pad, the length of antocular and postocular areas, antennae, rostrum, tibia, prothorax and foretibial, foretibial pad, egg and lateral oviduct indices are given in tables 6 and 7.

4. Discussion

The foregoing account on the biological, behavioural and morphological parameters of 85 species clearly demarcate the subfamilies Acanthaspidinae, Harpactorinae and Piratinae. Acanthaspidinae has the following characteristic features: moderately long incubation and stadial periods, eclosion and ecdysis at night, II or III stadium the shortest, females live longer than males, sex ratio slightly male biased, long preoviposition period, more number of egg batches with comparatively lesser number of eggs, fairly good hatchability, efficient prey capturing, nymphal cannibalism and nymphal congregational feeding in early instars, short premating period, mating acts similar to the acts of predation, short duration of copulation of side to side position, long time taken to eject the spermatophose capsule, frequent mating with short intervals, dull or warning coloured adults, curved rostrum, globose or oval eggs, well developed tibial pad, brown colour nymphs with tufts of stiff median and lateral abdominal hairs, scape and the first flagellar segments as the shortest and longest antennal segments, medial rostral segment as the longest, highest tibial index, intermediate tibial pad index, lowest egg index and moderate lateral oviduct index, eggs loosely laid but glued to excreta, nymphal camouflaging and fairly developed head nodding behaviour.

Harpactorine members have shortest incubation and stadial periods, shortest

stadiun mostly in the third instar, eclosion and ecdysis occur in the afternoon, male and female with equal longevity, female biased sex ratio, moderately long preoviposition period, less number of batches of eggs with more number of eggs, highest hatchability, poor prey capturing efficiency, quickly paralysing the prey, lifting the prey with rostrum, less sucking time with less number of sucking sites, nymphal congregational feeding in all instars, nymphal cannibalism in early instars, long premating and copulation period with a characteristic precopulatory riding, short time to eject the spermatophore capsule, fairly good frequency of mating with long interval of mating, alate, brightly coloured adults, ochraceous nymphs with straight and club shaped hair, elongate eggs, poorly developed tibial pads, slightly longer postocular area, pedicel the shortest and the scape or the II flagellar the longest segments of antenna, medial rostral segment the longest, mid tibia the shortest, poor fore tibial and fore tibial pad indices, highest egg index, poor oviduct index, elongate eggs laid in clutches and cemented together and to the substratum, death feigning and spitting behaviour.

Piratine reduviids have longest incubation and stadial periods, eclosion and ecdysis at night, short adult longevity, males live longer, female biased sex ratio, short preoviposition period, poor fecundity and hatchability, efficient and aggressive prey capturing, strongly developed nymphal cannibalism, aggressive mating behaviour with short duration of copulation, lowest frequency of mating with longest interval between mating, adults warningly coloured, brown or black nymphs, alary polymorphism, deeply curved rostrum, elongately oval eggs, highly developed tibial pad, longer anteocular area, scape the shortest and flagellar the longest antennal segments, medial rostral segment the longest, shortest tibial pad and lateral oviduct indices, eggs laid deep inside the soil, aggressive head nodding with 'crich-crich' sound.

The biological, behavioural and morphological parameters exhibited by these 3 subfamilies not only demarcate them from each other but also indicate the affinities in between them. Subfamily Acanthaspidinae is more closer to Piratinae and also intermediate in between Harpactorinae and Piratinae. This observation also lead to an understanding of their evolutionary origin. Livingstone and Ambrose (1984) traced the evolutionary origins of the subfamilies of Reduviidae and their observations very closely corroborate with the present study.

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ome aspects of biosystematics of Bruchidae (Coleoptera)

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Abstract. Bruchidae forms a small compact group of phytophagous Coleoptera with well defined ecological limits. Its members are by and large seed eaters, mostly attacking the seeds of Leguminosae including edible legumes. Several genera show definite host plant relationships and many species present strict host specificity. The bruchids of economic importance fall into two categories viz (i) store bruchids and (ii) field bruchids, with separate voltinism and distinct pattern of development. The bruchids of no recognized economic importance also have characteristic host plant relationship, pattern of distribution and avoidance of interspecific competition. Apart from the known taxonomic discrimination on the basis of external morphology, the genera and species can also be characterized from variations in the internal anatomy, the number and structure of chromosomes and the quality and quantity of their macromolecules. Behavioural peculiarities are also useful for the discrimination of species and genera. The time, manner and duration of copulation, site of oviposition and time and methods of emergence provide useful variations. Compatibility and incompatibility of various host seeds dependent on the amount of non protein aminoacids, antiprotease compounds, hemagglutinin fractions and other toxic substances for different bruchid species and the capability of the latter for combating the same also provide valuable demarcative information.

Keywords. Internal anatomy; chromosomes; behaviour.

. Introduction

t is well known that the conventional taxonomic procedure in different groups of nimals is primarily based on morphological characteristics that are stable and iscriminatory to facilitate identification and determination of suitable status for ifferent taxa. This procedure has been found by and large quite satisfactory and is ather difficult to replace. Accordingly, the identification of the species and their llocation to higher taxa will continue to be founded on the morphological features or a long time to come. This is so despite the fact that the category of species is now haracterized on biological criteria and that the higher taxa like genus and family lso show appreciable ecological, biochemical and genetical relationship among ifferent animal groups. However, scattered works on different life processes of many pecies have provided useful information in the areas other than the external mor-hology which can be profitably used as supplementary material for the confirma-on of taxonomic decisions and for solving complex problems of taxonomy dealing vith sibling species and infra specific categories. The true relationship among such axa is often shrouded by such evolutionary phenomena as convergence, parallel volution etc. In fact, the need for compilation and evaluation of ecological, iochemical, physiological and behavioural characteristics of the animal species has ncreased with the increase in the complexity of taxonomic challenges and this area is ast emerging as a distinct field of biosystematics. This communication is an attempt o review and compile the existing knowledge of the biosystematics of the family Bruchidae.

Bruchidae is rather a small family of phytophagous Coleoptera under which about 1300 species referable to about 70 genera have so far been recorded from different parts of the world. It is a compact group with poor genetic differentiation and high degree of interspecific similarity. The eggs are laid on the surface of the seeds, the larvae and pupae live within the seeds and the adults make their exit by pushing against thin caps or windows prepared by the mature larvae just before pupation. Whereas the larvae are voracious eaters, the adults hardly consume any substantial amount of food. The adults, commonly called as seed weevils, are small in size, measuring between 2–5 mm in length and the variation in their size is usually related to the size of the host seeds.

2. Observations

2.1 *Ecology*

The family Bruchidae is so evolved as to feed on the seeds of different plant families. Excepting some species which are associated with the seeds of families like Malvaceae, Convolvulaceae, Rosaceae, Papaveraceae and Palmae etc, about 90% of the species attack seeds of Leguminosae (Southgate 1979). Out of 5 subfamilies of Bruchidae, the largest Bruchinae is almost completely associated with Leguminosae while other 4 families attack seeds of non-leguminous plants. At the generic level also, there appears a strong pest-host correlation. Several genera such as *Abutiloneus*, *Megacerus* and *Caryotrypes* (Southgate 1979) are restricted to their respective host genera but genera like *Bruchidius*, *Callosobruchus* and *Conicobruchus* have wider ranges of host genera. A limited or varied host specificity is also witnessed at the level of species.

According to another ecological interpretation, the family Bruchidae can be divided into two major categories, viz (i) store bruchids and (ii) field bruchids (Southgate 1979). The store bruchids are obviously pests of edible seeds which are stored after the harvesting of the legume crops. The store bruchids are fast breeders, have short life cycles and life spans and, thus acquire the potentiality of passing a number of generations in the course of the year. With the food supply in plenty, the breeding is continous without any interruption and the fecundity is high. Such species mainly come from the genus *Callosobruchus* (Arora and Pajni 1957; Arora and Singh 1970; Pajni and Jit 1976) to be shared by a single species each from the genera *Zabrotes*, *Acanthoscelides* and *Bruchidius*. The field bruchids, on the contrary, include representatives of almost all the genera but include only a few species of economic importance. The field bruchids are, as a rule, univoltine and the single generation in the year is marked by long life cycle and long life span, with the intervention of a phase of imaginal diapause. Moreover these bruchids are usually host specific and have an obligatory requirement of feeding on the host flowers for attaining the capability of reproduction. Although this correlation has been verified only in a few species (Pajni 1981, 1984; Pajni and Mittal 1984), but is likely to prove as a general ecological principle for the field species in general. The host plants of the field bruchids however come from a number of genera without obeying any relationship with the bruchid genera. But a rearrangement of genera of both groups on the basis of biochemistry might project their real relationships.

2.2 *Behaviour*

Several bruchid species are characterized by such behavioural peculiarities as to make them distinctive from other species and are therefore important in their identification. One such character relates to their copulatory behaviour. The males and females copulate in one-above-the-other position and the male holds the female with the help of its fore and middle legs. However, *Callosobruchus theobromae* is exceptional as the male of this species does not hold the female during copulation (Chopra 1986). The time of copulation is also quite variable. Most of the species copulate at all hours of the day but pairing in *Caryedon serratus* takes place only at dusk time (Pajni and Mann 1979) and in *C. theobromae* in the darkness of night or in the early hours of the morning. A similar variation is seen in the duration of copulation, with *Bruchus* and *Callosobruchus* species and, *Zabrotes subfasciatus* having a short duration of less than 8 min (Arora 1977; Pajni and Sood 1984; Pajni and Jabbal 1986) and *Conicobruchus* and *Caryedon* mating for a much longer period of 20–30 min (Pajni and Mann 1979; Chopra 1986). *C. chinensis* is unique in showing the minimum copulation period of about 45 s (Arora and Singh 1970). The males of most species are polygamous but the capacity of male to inseminate the females varies a good deal (Pajni 1987). Similarly, *C. chinensis* and several field species are monoandrous but others show some degree of polyandry.

The identity of *Z. subfasciatus* can be established from the shape of its eggs which are spherical in comparison with the oval eggs of all other species. This species is also distinctive in laying eggs in clumps. The eggs are usually laid on the surface of the seeds or pods where they are cemented with the help of secretion. However *Bruchidius angustiforns* (Singal 1981) and *Conicobruchus indicus* (Chopra 1986) lay their eggs on calyces of the host flowers. Whereas *Neltumius texanus* (Johnson 1978) and *Sennius simulans* (Center and Johnson 1972) have their eggs attached with the help of anchoring strands radiating from the egg chorion, *Acanthoscelides obtectus* simply scatters its eggs among the harvested seeds (Southgate 1979), while other species of this genus insert them in the holes bitten in the outer tissue (Skaife 1926; Zachariae 1958).

The first instar larva in the family Bruchidae is different from the 3 subsequent larval instars in possessing well formed thoracic legs, an H-shaped plate on the prothorax and a hatching spine on each lateral side of Ist abdominal segment, marking some sort of hypermetamorphosis. Genus *Caryedon* is however characteristic in possessing well developed legs even in the mature larvae. In fact, the larvae of this genus and perhaps the entire subfamily Pachymerinae are exceptional in having long salivary glands, two pairs of malpighian tubules, 11 pairs of air sacs and in showing the habit of cocoon formation (Vats 1979). The number of eggs laid on each seed of the host depends largely on the density of the adults and is a poor indicator of the identity of the pest. However the number of adults emerging from a seed has a definite pest-host relationship and is mainly decided by the proportion between the size of the bruchid and the host seed. In this manner, many species like *Callosobruchus maculatus* (Arora and Pajni 1957), *Callosobruchus analis* (Pajni and Jit 1976), *Bruchus pisorum* (Pajni and Sood 1984), *B. lentis* (Pajni and Mittal 1984) and *C. theobromae* show the emergence of one adult from each infested seed. On the other hand, the number of adults that emerge from a single seed has been reported as 21 in the case of *Z. subfasciatus* (Pajni and Jabbal 1986), 8 in *Callosobruchus chinensis*

(Arora and Singh 1970) and 28 in the case of *Bruchus rufimanus* (Riley and Washington 1892) in their respective hosts of *Phaseolus vulgaris*, *Cicer arietinum* and *Vicia faba*. On the other extreme, there are several instances where the maturation of the larva to the adult stage requires the consumption of up to 4 seeds as reported in the case of *Conicobruchus* spp. attacking *Crotolaria* spp. (Skaife 1926; Prevett 1967; Chopra 1986) and *Sennius* spp. attacking *Cassia baubinioides* (Center and Johnson 1972).

Pupation generally occurs within the seed cavity, one edge of which has only the thin seed coat intact and is demarcated on the out side as a circular window or cap. The cap is subsequently incised along the margin and pushed out by the emerging adult. However, species consuming the entire or the major part of the seed prepare the cap on the surface of the pod as for example in *C. indicus* (Chopra 1986) and *Bruchidius saundersi* (Arora 1977). The larva of *C. serratus* prepares a papery cocoon for its pupa which emerges partially out of the host seed (Pajni and Mann 1979) or remains within the pod (Southgate 1979). In *C. theobromae*, the mature larva has to cut a hole in the seed as well as the adherent part of the pod (Chopra 1986) for the exit of the adult from unripe seed. Other species consume the seeds and the adults wait for the subsequent splitting of the pod for dispersal as in *Bruchidius ater* (Frankenhuyzen and Perquin 1972).

2.3 *Internal structures*

Apart from the special anatomical features in the subfamily *Pachymerinae* stated above, the subfamilies *Bruchinae* and *Amblycerinae* can also be characterized from the number of air sacs in their mature larvae which are 4 pairs in the former and 5 pairs in the latter (Vats 1972). In the adults also, a good amount of anatomical variation, particularly in the reproductive organs is witnessed. The testes in this family always comprise of two pairs of follicular structures and the number of follicles in each testis is highly variable, being 14–28 in *Caryedon* (Singh 1981), 1–18 in *Bruchidius* (Singh 1978a) and 8 in *C. maculatus* (Pajni 1968). Similarly, the number of male accessory glands also varies at the level of genera. There are 5 pairs of these glands in *Specularius*, 4–5 pairs in *Callosobruchus* and 4 pairs in *Conicobruchus*, *Bruchidius*, *Sulcobruchus* and *Zabrotes*, 3 pairs in *Bruchus* and *Caryedon* and the minimum of 2 pairs in *Spermophagus*. The histology, shape and position of the glands show differences at the species level (Singh 1978b). In the females, the number of ovarioles per ovary varies in different species. A low number of 3–5 ovarioles per ovary is present in *Amblycerinae* followed by 6 ovarioles per ovary in a majority of *Pachymerinae* and *Bruchinae* and ranging between 7–18 ovarioles per ovary in genera *Bruchidius*, *Conicobruchus* and *Bruchus* (Singh 1973). Studies on the karyology of bruchids show that in the subfamily *Pachymerinae*, the diploid number of chromosomes varies from 18–20 and is supposed to be primitive, followed by *Bruchinae* with diploid number of 20–22 chromosomes which rises to 26–32 chromosomes in *Amblycerinae* which is therefore the most specialized subfamily (Yadav 1973). Different species cannot be characterized by the number of chromosomes or sex mechanism but a few polytypic species like *Callosobruchus chinensis* have been reported to show different number of chromosomes in component sub species or strains (Takenouchi 1955, 1971; Sharma *et al* 1967; Yadav 1969, 1971). A similar type

of intraspecific chromosomal variation has been recorded in *C. maculatus* (Yadav 1971). The noted variation in the chromosome number of certain populations within a species is related to the appearance of morphologically expressed polymorphism in *Callosobruchus* species (Arora and Pajni 1957; Pajni 1986a, b, 1987). Different morphs have also been found to differ in their fecundity and month of appearance in the laboratory cultures.

2.4 *Biochemistry*

The suitability of a particular host for the attack of a bruchid species is determined by the biochemical composition of the seeds and the adaptive characteristics developed by the bruchids for tackling the toxic contents of such seeds. Accordingly, some legumes are incompatible or less compatible for the development of some bruchid species. This correlation decides the host range of various species of store bruchids leading to different degree of host specificity. The 3 common *Callosobruchus* species each can attack a wide range of edible legumes although the number and type of host seeds is different in each case. *Zabrotes subfasciatus*, on the other hand, has *P. vulgaris* as its major host but can also thrive on *Glycine max*. *P. vulgaris* is not attacked by any of the other store bruchids, showing their inability to neutralize the incompatible contents of these seeds. In spite of these limitations on their host range, the bruchids express a high degree of adaptive capacity to attack a variety of legumes in different parts of the globe. This is also supported by their successful breeding in the laboratory on such host seeds which other wise escape their attack in nature (Pajni 1986b).

Various species of store bruchids studied for the variations in their metabolic reserves also provide useful information for their characterization. According to Sharma and Sharma (1979a, b), the fresh females of *Z. subfasciatus* have 45·7% total lipids (TL) as compared to 36·9% of TL in *C. maculatus*. The corresponding figures for the males of these species are 38·2% and 32·3% respectively. These two species also differ in their body weight and water content, with the female of *C. maculatus* weighing two times more than that of the female *Z. subfasciatus* and the male of the former weighing 3 times more than that of the male of the latter (Sharma and Sharma 1984). There is however only a slight difference in the glycogen contents (Sharma and Sharma 1980) and tissue proteins (Sharma and Sharma 1980) in the two sexes of both the species.

It follows from the foregoing account that different species of bruchids can be easily distinguished on the basis of non-morphological characters pertaining to their ecology, behaviour, physiology and biochemistry. The morphs of the dimorphic/polymorphic species can also be similarly characterized. Such information is accordingly of great utility in strengthening the morphological basis of taxonomy and in solving its intricate problems.

3. Discussion

The foregoing review on the biosystematics of Bruchidae reveals that the members of this family form a well knit compact group and show striking similarity in their outer appearance. A correct identification of the species can be only confirmed from the

study of their male genitalia. Accordingly, the use of morphological criteria for specific determination is rather a difficult and laborious process. The bruchid species, however, can also be characterised on the basis of biological characteristics. Several species have a strict host plant relationship and can be easily made out from their respective host plants. The reproductive behaviour of bruchids is also highly variable and several species can be separated on the basis of time, manner and duration of copulation as well as from the condition of polygamy. Likewise, the time and pattern of oviposition is also characteristic in many species. The shape of the egg and the structure of the larvae present very useful discriminatory features as also the pattern of egg laying in these forms. The feeding behaviour of the larvae, the emergence of the imago and the number of adults emerging from a host seed are some other features in which different species show specific behaviour. The univoltine and multivoltine types of development vertically divide the family Bruchidae into two categories of field-bruchids and store-bruchids. A number of structural features of the internal anatomy show differences not only at the level of species and genera but also at the level of sub-families. These include number of air sacs and malphigian tubules in the larvae and the number of accessory glands, ovarioles and testicular follicles in the adults. The number of chromosomes show a definite correlation at the level of subfamilies and has been found to differ even in various morphs of the polymorphic species. The quality and quantity of macromolecules, which is dependent on the larval food, also shows significant variations in closely allied species. A study of all these aspects is of utmost importance for a complete understanding of the bruchid species and for their characterization in biological terms.

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Newer trends in the biosystematics of Membracidae

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Abstract. Membracids, in general, display a very few morphological characters of taxonomic value. Many species exhibit remarkable intraspecific variations, in addition to sexual dimorphism and cornuate polymorphism that makes it difficult to allocate them to their respective taxa, unless a long series of male and female individuals belonging to the same population are available for examination. The male genitalia, although of value in the diagnosis of higher categories of the Membracidae, find limited application at the species level. The nymphal characters, especially those of the fifth immature stage, are very useful in the identification of the species, but nymphal materials are not available except for a few species, and the host plants of many species remain unknown. In recent years, it has become imperative to consider the ecophysiological aspects of these insects for a better and precise understanding of their systematics. The host plant specificity of some species of membracids, the mutual association of a particular species of membracids with a particular species of ants and the parasite-host interaction involving certain chalcidoid egg parasites of membracids are considered with a view to evaluate their role in the membracid taxonomy. The impact of differential reproductive potential in terms of host diversity, the allochronic differences in mating and initiation of oviposition, and the differences in the relative growth patterns with particular reference to the polymorphic species *Oxyrhachis tarandus* (Fabricius) on 5 species is investigated.

Keywords. Membracidae; host-plant specificity; ant-attendance.

1. Introduction

Within the Membracidae relatively few morphological characters of taxonomic value are found at the higher category level (Deitz 1975). Even at the specific level adults of membracids, in general, display a very few morphological characters of systematic importance. Quite a number of species examined from India have been found to exhibit remarkable intraspecific variations added to sexual dimorphism. In view of the conspicuous development of pronotum and its processes, taxonomists have relied mostly upon the disposition and relative development of horns in the classification at generic and specific levels. This has led to misconception and errors in the identification of several species. Funkhouser (1917) stressed on the value of male genitalia in membracid taxonomy, and Caldwell (1949) attempted to revise the North American genera of Ceresini on the basis of male genitalia. However, a review of literature on this subject shows that although recent workers have placed increased emphasis on the genitalia, even the most extensive studies are of narrow scope (Deitz 1975). The male genitalia of membracids, from which so much was expected, have proved unsatisfactory, for although at a tribal level they are distinctive, they have proved so similar in general shape and so variable in microscopic details, that they can only be used as subsidiary characters (Capener 1962). Membracid nymphs display significant morphological characters of taxonomic value (Matausch 1910, 1911; Yothers 1934; Yothers and Allen 1941; Funkhouser 1951; Ananthasubramanian and Ananthakrishnan 1975; Quisenberry *et al* 1978). Nymphal taxonomy has the

added advantage, because the nymphs of even highly polymorphic species are remarkably uniform and monomorphic, displaying characters sufficient to distinguish them from related species. Unfortunately, nymphal materials are not available and are rarely represented in museum collections.

The present study aims at evaluating the role of host-plant specificity, the mutual relationship between a particular species of membracids and a particular species of ants, the ovipositing behaviour, the biogeographical influence on polymorphism and the impact of differential reproductive behaviour in some of the local species of membracids, following the methods of Ananthasubramanian and Ananthakrishnan (1975).

2. Host-plant specificity

The literature referring to hosts adopted by Membracidae from southern India is not extensive, and is confined to the observations made by Ananthasubramanian and Ananthakrishnan (1975) and supplemented afterwards by Ananthasubramanian (1979, 1984). The membracids confine themselves to very definite host plants both for feeding and for oviposition. In many cases the association between the membracid and the host is so characteristic that a knowledge of the one is sufficient for recognition of the other (Funkhouser 1917). In southern India, the more important natural orders of plants harbouring the membracids are Leguminosae, Solanaceae, Rubiaceae, Rhamnaceae and Malvaceae, while they seem to avoid some families such as Euphorbiaceae, Labiatae and Asclepiadaceae, but there are exceptions. It is noteworthy that the membracids avoid almost all the plants of monocotyledons.

Many species such as *Leptocentrus taurus* and *Otinotus oneratus* are decidedly polyphagous being encountered on host-plants belonging to widely different natural orders, while some are oligophagous found on plants belonging to a single natural order. However, it is strictly the monophagous species that exhibit absolute host-plant specificity that have considerable value in the biosystematics of the Membracidae (table 1). *Leptocentrus moringae* not only confines itself to *Moringa oleifera* from September to December, but it is the only species of membracid ever found on this host plant during this period. From June to August this host plant harbours another species, *Gargara hraswa*. It is noteworthy that the same species of host plant does not harbour two different species of membracids belonging to the same genus during the same period. Thus, *Oxyrhachis uncatus*, *O. krusadiensis* and *O. brevicornutus*, all of which appear to be monophagous, occur on *Acacia arabica*, but not at the same time on the same host plant. It is in respect of polymorphic monophagous species (such as *Leptocentrus varicornis* found only on *Zizyphus jujuba*) that a precise understanding of host-plant relationships would be useful in the allocation of the species to their correct taxa. In spite of their significance in the biosystematics of the membracids, the host records of many species are still unknown, because most collectors of membracids adopt beating and sweeping methods applied over wild shrubs and bushes without any consideration to the host plants.

3. Ant-attendance

Many species of membracids are attended by ants for the sake of the so-called honey-dew, an anal secretion of the former eagerly sought for by the latter, and in

Table 1. Host-plant specificity in some membracids from southern India.

Name of membracid	Name of host plant
Oxyrhachis minusculus Anan. and Anan.	*Casuarina equisetifolia*
O. krusadiensis Anan. and Anan.	*Acacia arabica*
O. brevicornutus Anan. and Anan.	"
O. uncatus Melichar	"
O. malabaricus Ananthas.	*Tamarindus indicus*
Lanceonotus cinnamomi Ananthas.	*Cinnamomum tamala*
Leptocentrus rhizophagus Anan. and Anan.	Prop roots of *Ficus bengalensis*
	Thespesia populnea
L. bauhiniae Anan. and Anan.	*Bauhinia tomentosa*
L. varicornis Anan. and Anan.	*Zizyphus jujuba*
L. moringae Anan. and Anan.	*Moringa oleifera*
L. mangiferae Anan. and Anan.	*Mangifera indica*
L. bajulans Dist.	*Casuarina equisetifolia*
L. major Anan. and Anan.	*Michaelia champaka*
Otinotus mimicus Dist.	*Agapanthus umbellatus*
Telingana nigroalata Anan. and Anan.	" "
T. consobrina Dist.	" "
Tricentrus spathodei Ananthas.	*Spathodea campanulata*
T. nobilis Ananthas.	*Boerhaavia repens*
T. albomaculatus Dist.	*Datura fastuosa*
T. congestus (Walker)	*Vernonia cinerea*
T. purpureus Anan. and Anan.	*Polygonum* sp.
Gargara pellucida Ananthas.	*Gymnosporia montana*
G. albitarsis Anan. and Anan.	*Tecoma stans*
G. rustica Anan. and Anan.	*Zizyphus jujuba*
G. malabarica Anan. and Anan.	*Phyllanthus emblica*
G. hraswa Ananthas.	*Moringa oleifera*
Parayasa nigrolimbata Ananthas.	*Gymnosporia montana*
Coccosterphus tuberculatus Motsch.	*Morinda tinctoria*

many instances the hiding places of the membracids are easily located by the presence of the attending ants. Since in many instances, a particular species of membracid is always associated with a particular species of ant, a precise knowledge of this trophobial mutualism would be of immense value in the identification of the membracid species involved. The same observations were made by Capener (1962) with reference to the African species of membracids. Each species of membracid in a particular locality is attended by the same species of ant throughout the year. For instance, the common species of gregarious membracid, *Oxyrhachis rufescens*, is regularly attended by the large ants of the species *Camponotus sericeus*, wherever this membracid colony occurs. *Crematogaster* sp., a small shining black ant, regularly attends on *Coccosterphus paludatus* Dist. and *Oecophylla smaragdina*, a ferocious reddish brown ant, attends on *Oxyrhachis krusadiensis* on its host plant, *Acacia arabica*, and on *Anchon ulniforme* Buckton on its host plant, *Cajanus cajans*. *Anoplolepis longipes*, a smaller reddish species of ant, is associated with *Leptocentrus bauhiniae* and on *Gargara rustica* on their respective host plants, *Bauhinia tomentosa* and *Z. jujuba*. *Myrmecaria brunnea* and *Meranoplus bicolor*, both characterised by backwardly directed spines, attend on *Leptocentrus rhizophagus* and *L. moringae* respectively. *Solenopsis germinata* var. *rufa*, a small brown ant nesting on the ground near trees, attends on *Tricentrus albomaculatus* on its host plant, *Datura fastuosa*. Colonies of a

large species of membracid, *Leptocentrus leucaspis*, are attended by a minute species of ant, *Paratrechina longicornis*. In those species of membracids that are multivoltine completing several generations in an year on the same host plant, the ant-attendance is found to be a regular feature. Thus, a knowledge of the host specificity of the membracids and the species of ants attending on them may be used as effective tools in the biosystematics of the membracids.

4. Biogeographical influence on the cornuate polymorphism in *Tricentrus pilosus* and its impact on membracid taxonomy

Ananthasubramanian and Ananthakrishnan (1975) reported the occurrence of cornuate polymorphism in several species of membracids from southern India. Of particular interest is this phenomenon found in *T. pilosus* A and A, an oligophagous species found on the prop roots of *Ficus bengalensis* and on the slender twigs of *Thespesia populnea* in and around Madras. The females of this membracid include 4 different morphs, viz (i) normal horns, (ii) short horns, (iii) vestigial horns and (iv) no horns. Males include 3 different morphs, viz (i) short horns, (ii) reduced horns and (iii) no horns. The frequency of occurrence of the different morphs of this species on its two host-plant species was reported by Ananthasubramanian (1979). Recently, the discovery of geographical races of this species has added new dimensions to the complexity of this species complex (figure 1). Specimens collected from Chedia Tope (Andamans) on *T. populnea* and prop roots of *F. bengalensis* were found to be remarkably uniform and monomorphic, but in both sexes the horns were distinctly longer than those of the females with normal horns collected from Madras, while those collected from Narakkal (Kerala) on the same species of host plants, though monomorphic, showed horns shorter than those of normal females collected from Madras. These observations have led to the conclusion that biogeographical factors should have a decisive role in bringing about phenotypic plasticity within certain limits, and hence great caution has to be exercised in the matter of allocating the different morphs to their correct taxon. Mating experiments performed on the different morphs collected at Madras from their host plants showed the absence of reproductive barrier, but the eggs deposited by such females failed to develop.

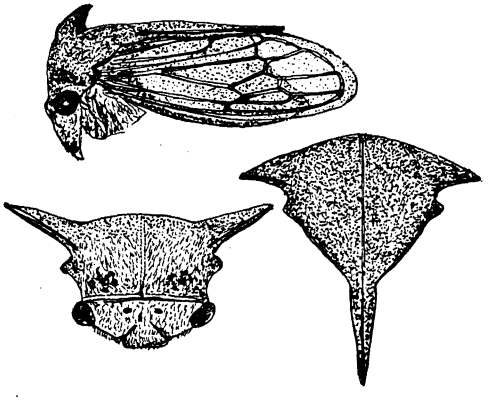


Figure 1. Geographical form of *T. pilosus* from Andamans.

Intercrossing of the morphs from different geographical regions has not been successful due to the difficulty of maintaining the insects alive on holidics.

5. Ovipositing behaviour

Though the eggs of different species of membracids are more or less identical in shape and lack sculpturing of any kind, the ovipositing behaviour is distinctive, constituting reliable parameters in the biosystematics of the family. In all the species of the Subfamily Oxyrhachinae with no known exception, the eggs are deposited on fairly thick twigs and are arranged in a palmate manner, the opposite rows converging at one end and diverging at the other. In fact, this is the pattern of oviposition characteristic of all the species of the only genus *Oxyrhachis*, the different species differing in the number of eggs deposited in each egg mass. Curiously, the egg mass of *O. brevicornutus* is covered by a viscid secretion of the female. In the Subfamily Centrotinae the ovipositing behaviour is much varied. In the Leptocentrini, the eggs are always arranged in straight rows. The oviposition slit, however, varies in the different species of *Leptocentrus*. In *L. taurus* F. for instance, two egg slits are cut by the ovipositor side by side and eggs are deposited in two parallel rows. The oviposition wound does not completely heel up so that a deep elliptical scar is left behind. *L. rhizophagus*, *L. moringae*, *L. bauhiniae* and *L. major* deposit their eggs in a single straight row, and the slit closes by springing back, leaving an extremely narrow streak. The length of this streak which is species-specific, is correlated with the number of eggs laid. In *L. leucaspis*, 55–70 egg slits, each measuring 3–4 mm long, are laid close to each other. In the genus *Otinotus*, exemplified by *O. oneratus* (Walker) the eggs are laid in irregular manner very superficially, and the different rows of eggs overlap to a considerable extent. *Gargara albitarsis* and *Telingana nigroalata* lay their eggs solitarily. *Gargara mixta* and *G. extrema* make numerous short straight or crescentic slits very close to each other; the slits are deep so that the eggs are almost completely enclosed. In the tribe Coccosterphini the ovipositing behaviour differs in different genera. In the genus *Coccosterphus* the egg slits are invariably made in tender twigs of herbs or shrubs, while *Parayasa* invariably oviposit on the herbaceous plants which it inhabits. The egg clusters are short, each containing a small number of 3–5 eggs in the former genus and 5–9 in the latter, and the egg masses are laid far apart. In *Eucoccosterphus* the eggs are laid either on woody twigs or on the peduncles, the egg masses being close to each other, each containing 4–9 eggs.

6. Allochronic shifts in oviposition in the intraspecific variants of the polymorphic species complex, *Oxyrhachis tarandus* (F.)

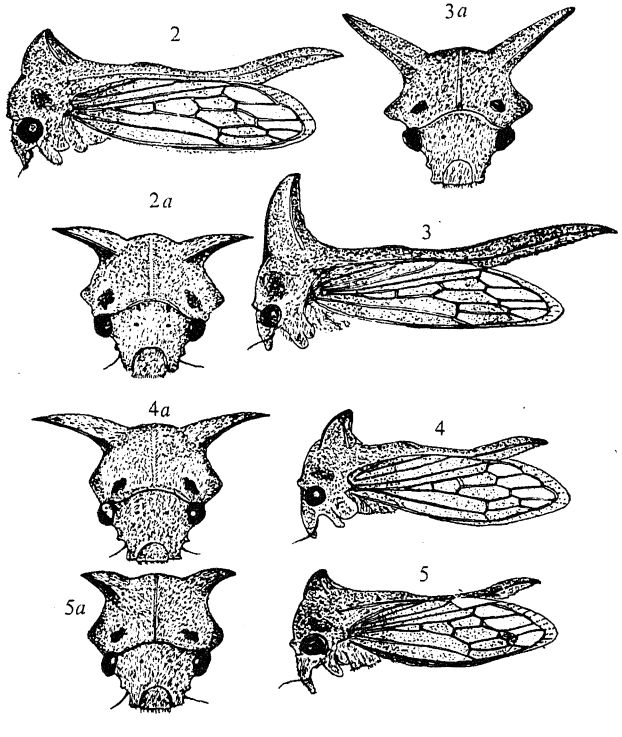
An analysis of the morphometric variations in *O. tarandus* collected from 5 of its host-plant species, viz *Prosopis spicigera*, *A. arabica*, *Pithecolobium dulce*, *Peltophorum ferrugineum* and *Poinciana regia*, disclosed 4 groups of individuals with reference to differences in the degree of development of the suprahumeral horns and the disposition of the posterior process. These 4 groups are: (i) Individuals with moderately developed suprahumeral horns which are less divergent; the posterior process moderately elevated and extending slightly behind the tegmina, (ii) individuals with

suprahumeral horns longer and more divergent than those of group (i) and projecting obliquely upwards; width across tips of horns greater than that of group (i); posterior process moderately elevated as in group (i), but extending well behind the tegmina, (iii) individuals with suprahumeral horns about as long as those of group (ii), but never projecting obliquely upwards, the angles of their carinae being more or less similar to those of group (i); posterior process shorter, less elevated and never reaching the tips of the tegmina, (iv) individuals with suprahumeral horns much reduced; posterior process as in group (iii) (figures 2–5).

The egg masses of these 4 groups of intraspecific variants also showed differences in the number of eggs (table 2). The identification of the egg masses of 4 groups was made possible since the egg masses were guarded by the mothers by sitting on them for hours or for days.

It was also noticed that the initiation and termination of oviposition by the females of the 4 groups do not always occur simultaneously, but vary by several days or weeks. While oviposition has been noticed to occur in the case of group (i) individuals almost throughout the year, it occurs only from August to March in group (ii) individuals. In groups (iii) and (iv) individuals, initiation of oviposition occurs in the first and second (or third) week of October respectively, and the oviposition period extends to only $2\frac{1}{2}$–$2\frac{3}{4}$ months respectively (table 3).

The frequencies of occurrence of these 4 groups of individuals of *O. tarandus* are also found to differ on its 5 species of host plants analysed. On *P. spicigera* all the 4



Figures 2–5. Cornuate polymorphism in the species complex *O. taranaus.*

Table 2. Morphometric variations in 4 groups of individuals in the species complex of *O. tarandus*.

	Group I		Group II		Group III		Group IV	
	Female	Male	Female	Male	Female	Male	Female	Male
Length from frontal margin to tips of tegmina	8·0	7·4	8·2	7·5	6·8	6·4	6·8	6·5
Length from frontal margin to tip of posterior process	8·5	8·0	9·1	8·9	6·2	6·0	6·2	6·0
Width across tips of supra-humeral horns	4·5	4·2	5·2	3·0	5·2	5·0	4·9	3·5
Width across tips of humeral angles	3·1	2·4	3·3	3·1	3·12	3·0	3·2	3·1
Width across eyes	2·4	2·2	2·4	2·2	2·43	2·3	2·45	2·35
Length of horn	2·4	2·3	2·8	2·5	2·22	2·0	1·05	1·10
Width across bases of horns	1·25	1·19	1·4	1·35	1·2	1·9	1·26	1·9

Numbers indicate the mean value of 5 individuals in each group and in each sex. Measurements are in mm.

Table 3. Number of eggs per egg mass and the allochronic variations in the initiation and termination of oviposition in 4 groups of intraspecific variants in the species complex of *O. tarandus* during 1985–86.

	Group I	Group II	Group III	Group IV
Number of eggs per egg mass	80–112	92–118	63–78	34–40
Initiation of oviposition	2nd week of July	1st week of August	1st week of October	2nd or 3rd week of October
Termination of oviposition	1st week of June	4th week of March	1st week of January	1st week of January
Ovipositing phase	11 months	7 months	$3\frac{1}{4}$ months	$2\frac{1}{2}$–$2\frac{3}{4}$ months

Table 4. Frequency of occurrence of 4 groups of intraspecific variants in the species complex of *O. tarandus* on 5 of its host plants during the year 1985–86.

	P. spicigera	*A. arabica*	*P. ferrugineum*	*P. dulce*	*P. regia*
Group I	46–50%	20–25%	—	—	—
Group II	20–25%	40–45%	90–95%	—	7·5%
Group III	20–28%	—	5–10%	70–85%	2·5%
Group IV	10–14%	25–34%	—	15–30%	90%

groups of individuals occur, although group (i) individuals constitute 45–50%. On the other 4 species of host plants, one or two groups of the individuals are absent. Group (iii) individuals are not found on *A. arabica*, groups (i) and (iv) on *P. ferrugineum*, groups (i) and (ii) on *P. dulce*, and group (i) individuals are absent on *P. regia*. On *A. arabica* the frequency of occurrence of group (ii) individuals is higher than the group (i) and group (iv) individuals, while on *P. ferrugineum* group (ii) individuals form 90–95% of the total population. On *P. dulce*, group (iii) individuals form about 85%, while on *P. regia* group (iv) individuals constitute about 90% from November to February, after which these as well as the other groups of individuals disappear since this plant sheds off its leaves (table 4).

7. Discussion

The trophobial mutualism between membracids and ants, apart from its value in the biosystematics of the Membracidae, is significant in the context of evolutionary divergence. According to Wood (1982), ant-attendance on gregarious species of membracids enhances the survival rate of the membracid nymphs. Survival of nymphs varies among trees due to variation in attendance by ants. Variation among individual trees in nymphal survival and ant attendance promotes distinct insect distributions. Such distributions magnify microgeographic disjunct among insect populations on conspecific host-plant species and have facilitated the divergence of the species into a complex of reproductively isolated species, one on each species of the host plant. This concept of genetic differentiation along host plant lines, originally offered to account for the host plant races in the sympatric species complex, *Enchenopa binotata* Say by Wood (1980, 1982), Wood and Guttman (1981) and Guttman *et al* (1981) may be extended to account for the differences in the percentage of occurrence of the 4 groups of intraspecific variants of *O. tarandus* on its 5 host-plant species where they are attended by a large number of *C. sericeus*. Further studies in this direction are required to ascertain the role of ants in promoting disjunct insect distributions and sympatric speciation as the ultimate product of evolution.

Allochronic shifts in life-histories or adoption of new host plants by phytophagous insects have been implicated in the formation of host-plant races and reproductive isolation. Bush (1975) suggested that shifts in host plants promoted sympatric divergence in some phytophagous insects. Wood and Guttman (1981, 1982) have presented considerable data to support a sympatric model of speciation in the species complex, *E. binotata* by shifts in host plants. Studies on the origin of insect host-plant races and the evolution of species in certain phytophagous insects by Bush (1969, 1975), Alexander (1968), Tauber and Tauber (1977a,b) and Edmunds and Alstad (1978) show that new host races and species may arise sympatrically. The species complex, *O. tarandus*, offers a good opportunity to study the evolution of host plant races. Funkhouser (1951) considered the centre of membracid distribution to be tropic with subsequent migration to northern and southern regions. *O. tarandus* is a tropical species being distributed all over the Oriental realm, and it is predominantly polyphagous and multivoltine. Its adoption of certain host plant species which are deciduous, made it to adapt itself to the phenology of the host plants. Consequently, the number of generations completed per year had decreased on these host plants. Allochronic shifts in life-history patterns such as the differences in the initiation and termination of oviposition, and differences in the number of eggs in the egg masses—an index of the differential reproductive potential of this species are different in terms of host diversity—occurred in the individuals on different species of host plants. These changes were accompanied by morphometric variations particularly in the degree of development of horns and posterior process in the 4 groups of individuals adapted to a particular species of host plants, contributing to the initial stages in sympatric speciation. The present observations on *O. tarandus* are of a preliminary nature, and have to be confirmed by host and mate selection experiments in order to establish the distinctiveness of each group or host plant race with regard to their mating and oviposition behaviour, egg hatch, duration of egg and nymphal stages, nymphal mortality and adult survival, all these being the non-random factors leading to evolutionary divergence.

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Significance of haemolymph protein patterns in biosystematic studies of some grouse locusts (Tetrigidae: Orthoptera)

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Abstract. The grouse locusts (Tetrigidae) represent a group of small sized Caeliferan Orthoptera. These are considered to be primitive orthopterans related to Tridactylids on one hand to Acridids on the other. This family is represented in Indian subcontinent by about 90 species belonging to 35 genera. In the present study haemolymph protein profiles from 7 species from this family have been studied as an additional parameter to understand interrelationship amongst them. It is noted that the species *Paratettix dorsifer* and *Euparatettix personatus* belonging to the same subfamily Tetriginae show remarkable similarity in the haemolymph protein profile, thus justifying their classical taxonomical grouping. In case of subfamily Scelimeninae, however, of the 4 species studied 3 species viz *Eucriotettix flavopictus*, *Criotettix latifrons* and *Thoradonta pruthii* show a marked similarity, however *Eucriotettix harpago* shows a pattern closer to that observed in the subfamily Cladonotinae. This implies that though by classical taxonomic criteria the 4 species are close to each other this may not be a very natural grouping.

From this observation it becomes clear that help from additional parameters like protein profile studies, immunochemical studies, cytogenetic analysis, etc will prove to be very valuable for the real understanding of phylogenetic interrelations and evolution of this family in particular and the status of grouse locusts vis-a-vis the orthopteran insects in general.

Keywords. Haemolymph proteins; electrophoretic analysis; grouse locusts: Orthoptera.

1. Introduction

In the study of systematics of any group of organisms the classical and favourite approach for understanding its evolutionary and phylogenetic relationships, the most customary approach has been to resort to the morphological/anatomical methods. These represent the structural parameters. This 'museum' taxonomic approach is further variously supplemented by zoogeographical, geologically durational and even etho-ecological observations in interpreting the relationships. Undoubtedly, although such a practice is useful, sound and commonly adopted even today, it is not without certain limitations. It is for this reason, of late, that the various supplementary non-structural methods are being used. For example, the genetical, tissue-cultural and above all biochemical and immunochemical ones are being increasingly used as additional parameters to justify the phylogenetic relationships. Therefore, biosystematics can now be called a truely interdisciplinary approach with basic and applied potential.

Like in many other animal groups, insects can also be used in biosystematics. However, insects in general and Orthopterans in particular have been studied from such angles here and there (Blackith and Blackith 1968; Leone 1947; White 1951) only. However, as far as biochemistry of insect plasma/haemolymph proteins is concerned a first ever exhaustive review by Wyatt and Pan has appeared as late as in

1978. Herein it is seen that most of the work relates to the functional aspects of proteins.

At this Centre work is being done for quite sometime on grouse locusts (Tetrigidae: Orthoptera) covering etho-ecological, cytological (Godbole and Paranjape 1977; Paranjape *et al* 1987) and biochemical aspects with special reference to taxonomy and phylogeny of this interesting group of primitive orthopterans. The present paper attempts, by analysing haemolymph protein profile patterns of grouse locusts in particular, to evaluate the classical biosystematic status of this group.

2. Materials and methods

2.1 *Materials*

The insects used for the haemolymph protein studies were 7 species of grouse locusts (Fam. Tetrigidae), 1 species of grasshopper, viz *Chrotogonus* sps. (Fam. Pyrgomorphidae) and 1 species of cockroach, viz *Periplaneta americana* (Fam. Blattidae). Of the 7 species of grouse locusts *Potua sabulosa* belongs to the subfamily Cladonotinae; *Euscelimena harpago*, *Criotettix latifrons*, *Eucriotettix flavopictus* and *Thoradonta pruthii* belong to the subfamily Scelimeninae, while *Paratettix dorsifer* and *Euparatettix personatus* are included in the subfamily Tetriginae of the family Tetrigidae.

Excepting *P. sabulosa*, *E. flavopictus* and *T. pruthii* that were collected at Mahabaleshwar all other insect specimens were collected locally. Prior to their use for haemolymph protein separation the insects were maintained in the insect cages in the laboratory by simulating their natural habitat.

2.2 *Separation of haemolymph for electrophoresis*

0·1 ml of phosphate buffered saline (PBS) (with phenylmethylsulphonyl fluoride) was taken in an Eppendorf tube (1·5 ml). Another Eppendorf tube (0·5 ml) carried a small opening at the basal tip and was introduced into the 1·5 ml Eppendorf tube containing PBS. The hind leg of insect was cut at the base of the coxa and the insect was immediately put into the smaller tube, with its head pointing downwards. The larger tube was then capped. The tube was then centrifuged at 12,000 rpm for 10 min using Remi Micro Centrifuge RM 12C.

The inner tube was carefully removed. The haemolymph collected in the outer tube was maintained at 4°C till further use. This sample was later used for loading on the gel for electrophoresis. By this procedure sufficient haemolymph without cellular elements and other contaminants was available from a single insect.

2.3 *Electrophoretic separation of haemolymph proteins*

Polyacrylamide gel electrophoresis was employed to separate haemolymph proteins. The method used was according to the procedure described by Plummer (1977) and Work and Work (1972). A 7·5% gel system was employed with Tris-Glycine buffer pH 8·9. Gels were cast in glass tubes (10 cm long, 0·5 cm inner diameter). After

polymerization, preelectrophoresis was carried out for 30 min. The haemolymph samples (40 μl each) were loaded on the gels along with bromophenol blue (BPB) as marker dye. Electrophoresis was carried out, with a current 2 mA per tube, till marker dye reached the end of the gel. The gels were stained by Coomassie Blue for 1 h and then destained overnight.

2.4 *Calculation of percent relative electrophoretic mobility of protein bands*

Each protein band was assigned a value to denote its relative electrophoretic mobility as compared to the marker dye. For each tube mobility of the dye was equated to 100. Thus the per cent relative electrophoretic mobility (PREM) for protein bands was derived using the formula:

$$\text{Percent relative electrophoretic mobility} = \frac{\text{Distance (mm) travelled by a band from top of gel}}{\text{Distance (mm) travelled of BPB from top of gel}} \times 100$$

This information on the total number of bands on their PREM was used to compare the protein profile in different species.

3. Results

The observations made on the electrophoretic profiles of haemolymph protein from 7 species of grouse locusts, *Chrotogonus* sps. and *P. americana* are diagrammatically represented in figure 1. Total number of haemolymph protein bands observed in a species and the relative per cent electrophoretic mobility values calculated for each band are given in table 1. It is observed that the total number of protein bands vary between 6 and 13. Every species studied shows a set of protein bands varying in number from 3–6, which move considerably slowly (less than 10% REM). It is also seen that slow migrating bands are quite sharp and narrow as compared to some of the faster moving bands. This implies that the slow migrating proteins may be relatively less in concentration than the faster moving proteins in the haemolymph.

4. Discussion

The classical taxonomy on the basis of the structural and palaeontological information suggests certain affinities and phylogenetic relationships amongst the orthopteroid insects. An attempt has also been made to add to these the usefulness of etho-ecological characteristics with particular reference to the tetrigids (Paranjape *et al* 1987). As far as the Orthopteroid insects are concerned the classical approach can be briefly summarized as follows: (a) The cockroaches (subord. Blattodea = Blattaria) arose from Protoblattoidea (Carboniferous to Jurassic). The latter are either considered allied to or even part of the Protorthoptera that gave rise to Orthoptera (upper Carboniferous) represented by long and short horned grasshoppers, locusts, grouse locusts, crickets and so on. (b) Although cockroaches were formerly included in Orthoptera, these together with the mantids and on the basis of distinctive characters are now included in a separate order, viz Dictyoptera. In other words these are now considered as a natural group, not only from Orthoptera but

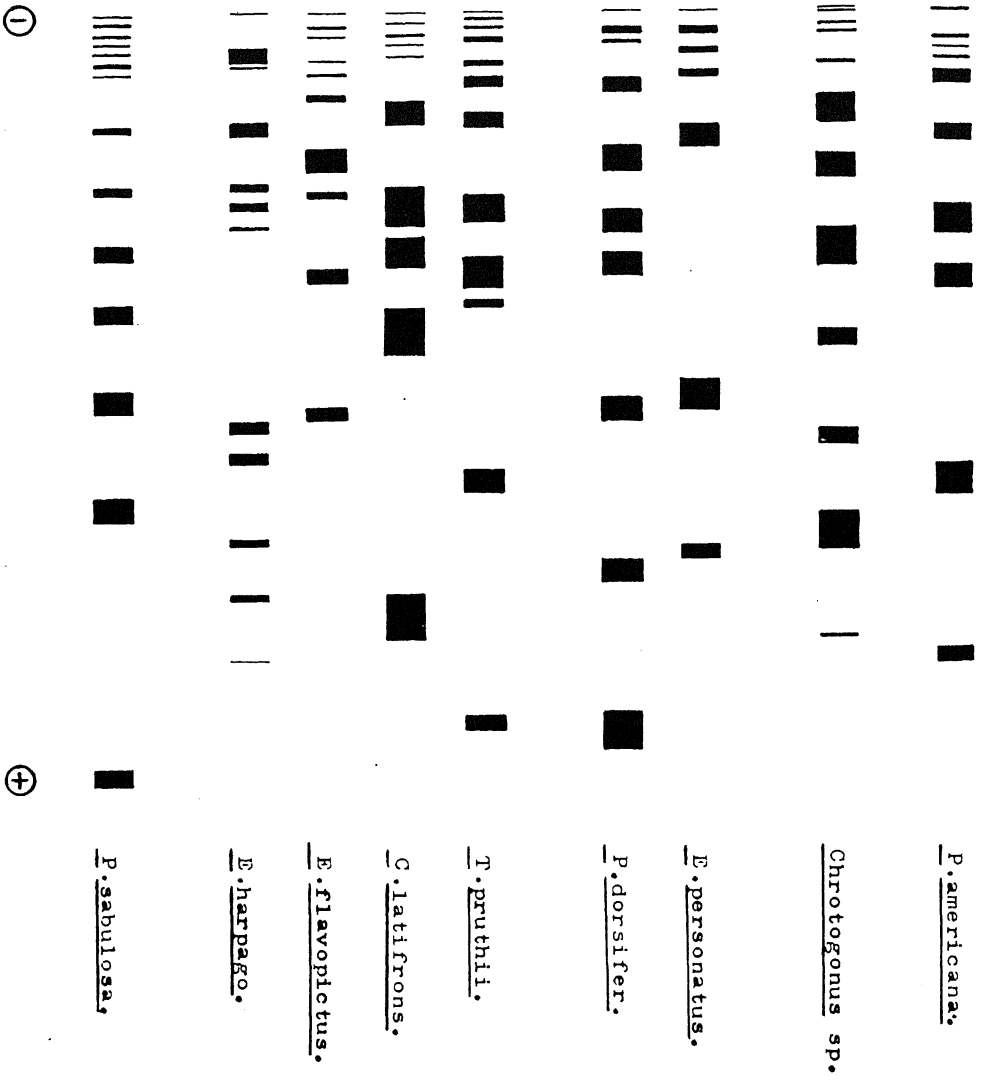

Figure 1. Diagrammatic representation of electrophoretic separation patterns of haemolymph proteins.

also from the Saltatorial Orthoptera and are considered closer to another Orthopteroid order, viz Isoptera. However, even today, cockroaches and grasshoppers can be considered related to one another as Orthopteran insects in an extremely broad sense of the term and their respective orders are definitely included in Orthopteroid insect orders. (c) Amongst Orthoptera and especially so in the Saltatorial Orthoptera the grouse locusts belonging to Tetrigidae are considered primitive and related to Tridactylidae (Pygmy mole and sand crickets) on one hand and Acrididae (short-horned grasshoppers and locusts) on the other (Hubbell 1978). (d) Of the 3 subfamilies from the family Tetrigidae studied by us the Cladonotinae seems to be the primitive,

Table 1. PREM of haemolymph protein bands.

Species \ Band No.	1	2	3	4	5	6	7	8	9	10	11	12	13
P. sabulosa	1·35	2·70	4·05	5·13	6·75	8·10	14·85	22·95	31·05	37·80	49·95	63·45	95·85
E. harpago	5·56	6·95	15·29	22·24	25·02	27·80	52·82	56·99	68·11	75·06	83·40		
E. flavopictus	1·56	3·12	6·24	7·80	10·92	18·72	23·40	34·32	51·48				
C. latifrons	1·41	2·82	4·23	5·64	12·69	25·38	31·02	45·12	77·55				
T. pruthii	1·16	1·74	3·48	5·80	9·28	13·92	25·52	33·64	37·12	60·32	91·64		
P. dorsifer	2·70	4·05	9·45	18·90	27·00	32·40	51·30	71·55	91·80				
E. personatus	2·66	5·32	7·98	15·96	49·21	69·18							
Chrotogonus sps.	0·67	1·99	2·66	6·65	13·30	19·95	30·59	42·56	54·53	66·50	79·80		
P. americana	3·69	4·92	6·15	8·61	15·99	27·06	34·44	60·27	82·41				

with the Scelimeninae having an intermediate position and the Tetriginae related more closely to Acrididae.

The current studies on the haemolymph protein patterns in Orthopteroid insects such as cockroach, grasshopper and grouse locusts generally support the above mentioned phylogenetic relationships. (i) It is observed that the haemolymph protein profile in a cockroach and in a grasshopper shows considerable similarity. (ii) The 7 species of grouse locusts, all representing the family Tetrigidae, show more or less similar profile where the PREM varies between 0·67 and 95·85. (iii) The only member of the subfamily Cladonotinae, viz *P. sabulosa* shows maximum number of bands and also the fastest moving band. (iv) Among the 4 species from the subfamily Scelimeninae, 3 species viz *E. flavopictus*, *C. latifrons* and *T. pruthii* show remarkably similar protein profile, while the fourth species *E. harpago* shows considerable deviation from the remaining 3. (v) Two species included in the subfamily Tetriginae, viz *P. dorsifer* and *E. personatus* show a remarkably similar protein profile.

These observations can be utilized to analyse the classical taxonomical organization of these species at the subfamily level. Placement of *P. dorsifer* and *E. personatus* in the same subfamily is supported clearly by very similar protein profiles. As far as the 4 members of the subfamily Scelimeninae are concerned, it is observed that haemolymph protein profile of *E. harpago* differs from the remaining 3 species. In addition, this protein profile seems closer to that observed in the member of the subfamily Cladonotinae. From this it may be concluded that inclusion of *E. harpago* in the subfamily Scelimeninae may not be a very natural grouping, but it may be placed separately in a group closer to Cladonotinae. Thus, it is clear that haemolymph protein profiles will prove to be very valuable additional parameter for the real understanding of phylogenetic interrelationships and evolution of this group.

From the point of view of cytogenetics, the Orthopteran group has been studied extensively. In the present case, the chromosome complements of the males of the species show distinct groups, one with $2n = 13$ chromosomes and the other with $2n = 17$ chromosomes, as far as the grouse locusts are concerned. The species with 13 as male diploid chromosome numbers are *P. sabulosa*, *E. harpago*, *T. pruthii*, *P. dorsifer* and *E. personatus* and those with 17 are *E. flavopictus* and *C. latifrons*. This information alone by itself is only of a limited value from the viewpoint of taxonomic studies. Additional studies such as chromosome banding, genomic DNA characterization, etc are required to apply them in studies related to establishment of

taxonomic interrelationships. Thus in the present situation, no correlation is established between protein profiles and karyotype.

As an extension to the biosystematic work using haemolymph protein profiles, it is proposed to undertake immunochemical studies on this group with a view to probe further into the affinities and the phylogenetic relationships at subgeneric and species levels. Studies on specific proteins/enzymes for electrophoretic variations are also undertaken as these would provide additional information from the point of view of establishment of interrelationships.

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Biosystematics of fig wasps (Chalcidoidea: Hymenoptera)

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Abstract. The fig wasps form a heterogenous group of microhymenopterans showing convergent evolution in response to adaptations for life in their unique habitat—the interior of the syconia of the different *Ficus* species. Sexual dimorphism is very common among the various tribes of the chalcidoid families constituting the fig wasps. This is in response to the different types of functions that the female and the male sexes are called upon to perform in their specialized habitat. The unisexual variations commonly met with in the male sex and rarely in the female sex, in the cleptoparasitic tribes belonging to the *Torymidae* and the *Pteromalidae* may be attributed to competitive feeding on a limited provision of food—a trophic factor. In the homeomorphic sexes of the subfamily *Epichrysomallinae* (*Pteromalidae*) copulation takes place outside on the top of the syconia. The importance of making observations on the biology including mating behaviour of the dimorphic (apterous and alate) males in the subfamily *Sycophaginae* (*Torymidae*) for the proper study of the systematics of this group is stressed.

The true fig wasps which are the fig pollinators (*Agaonidae*) are generally highly host specific, i.e. only one species of these pollinators is associated with a given species of *Ficus*. The *Ficus* species and its wasp pollinator are in intimate symbiotic relationship. There has been co-evolution of the *Ficus* species with its specific wasp pollinator. The females of these pollinator wasps often possess pollen storing structures as well as the behavioural attributes for the active collection of pollen from the stamens of the ripe fig and for the active dispersal of pollen on to the stigmas of the ovaries of flowers of tender figs into which these females penetrate for oviposition.

The ecological niche of the *Ficus* syconia is virtually identical to that of many plant galls and as such attract many species of gall-inhabiting *Eurytominae* (Family *Eurytomidae*). Such *Eurytominae* include a variety of genera, with numerous species both of phytophagous and entomophagous habits.

The modern systematics of the fig wasps is based on the above aspects of their functional morphology, biology, convergent evolution, sex-limited variations, sex-limited dimorphism, host specificity, behavioural attributes, etc. A conspectus of the modern classification of the fig *Chalcidoidea* is presented.

Keywords. Fig wasps; chalcidoidea; biosystematics.

1. Introduction

Classical systematics based on the study of only morphological features of organisms and built on the highly static type concept, over the years developed a reputation of being a matter of sterile pigeon holing (Hanson 1958). Effective research and action programme in basic and applied biology demand a sound taxonomic base (Lattin and Riley 1984). This is provided for in the newer trend in systematics or what is called biosystematics. Biosystematics is based on data derived not only from the study of morphology but necessarily also on studies of physiological, biochemical, eco-behavioural and genetic features of organisms as well as from their biometric analysis. Thus biosystematics is a dynamic area of study and research. The study of insect biosystematics has become increasingly significant in explaining problems which go beyond the classical systematic approach (Howse and Clement 1981) and

which are often of great importance in various aspects of agriculture, medicine and forestry.

Several genera and species of microhymenopterans belonging to families *Agaonidae*, *Torymidae*, *Pteromalidae* and *Eurytomidae*, because they develop in the receptacles of figs (*Ficus* spp.), form a heterogenous group—commonly called as fig wasps. Of the different families, the agaonids alone are true fig wasps in the sense that they lead a true symbiotic life and are responsible for pollination in *Ficus*. Others live either as parasites (torymid and pteromalid fig wasps) or as inquilines (eurytomid fig wasps). Owing to the unique habitat these wasps share, they show remarkable convergent evolution. Almost all species of fig wasps are characterised by pronounced sexual dimorphism: this is in response to the different functions that the male and female sexes are called upon to perform in their specialised habitat. The prevailing ecological niche—the ovary of the female flowers in the fig syconia—is more or less similar to that of many plant galls and as such attract many species of gall-inhabiting *Eurytomidae*. Fig wasp systematics to be sound and authoritative, should not be merely a descriptive compendium of morphological features, but should also effectively take into consideration the various aspects of their life such as functional morphology, biology, host-specificity, eco-behavioural attributes, sexual dimorphism, unisexual variations, unisexual dimorphism, convergent evolution, etc.

2. Review of literature

Though taxonomic descriptions of several species of fig wasps are available, only a few workers have attempted to study the biology and phylogeny of the group. Among these, the works of Grandi (1920, 1921, 1925, 1929, 1930, 1959 and 1961), Joseph (1953–1956, 1958, 1959a,b, 1966, 1984), Abdurahiman and Joseph (1976, 1978a,b, 1979), Wiebes (1963, 1973, 1976 and 1982–1986), Galil and Eisikowitch (1968, 1969, 1971), Galil *et al* (1973), Hill (1967) and Boucek *et al* (1981) are important. Their contributions have thrown light on the intricate relationships that exist between figs and fig wasps, the wasp adaptations—morphological, physiological and behavioural—involved, their reproductive strategies, life-cycles, co-evolution, phylogeny, etc. In this paper an attempt has been made to correlate the morphological, biological and behavioural attributes of this peculiar group of insects to their systematic affinities and diversities.

3. Observations

3.1 *Biological features*

A coordination of the developmental periods of the wasps and figs is a vital factor in the fig–fig wasp symbiosis. This has, in the course of evolution, caused extreme plasticity of adaptive mechanisms that are essential for the survival of both participants in the symbiosis. The success of this symbiosis is assured by the imposition of the developmental rhythm of the wasp with that of the plant partner. The viability and maturity of the unpollinated syconia, due to occupation by the wasp, is of utmost importance for the completion of the developmental cycle of the wasps.

At the early stage of development of the fig, the oviposition by the agaonid prevents the dropping of the tender figs and ensures the development of the figs to

final ripening. Longo (1909) and Leclerc du Sablon (1906) showed that as a result of oviposition by the agaonid, *Blastophaga psenes*, into the ovary of its host *Ficus carica*, the secondary nucellus is induced to divide, leading to the parthenogenetic development of the endosperm which serves as provision of food for the developing stages of the insect. It is quite likely that the determinant of this excitation for division is contained in the acid gland of the agaonids (Grandi 1920, 1961; Joseph 1958; Abdurahiman and Joseph 1978a,b). Joseph (1958) and later Abdurahiman and Joseph (1978a,b) found that the Pteromalid, *Philotrypesis* spp. and the torymid, *Apocrypta bakeri*, breeding in the receptacles of *F. carica* and *F hispida*, are subjected to partial larval starvation as a result of their cleptoparasitic mode of life on their agaonid hosts. Both the larvae of the agaonid and the pteromalid/torymid develop simultaneously up to their second instars inside a given *Ficus* ovary, feeding on the provisions made available by the agaonid female for its own larva. But as the quantity of the food left over at the disposal of the trophically competing larvae get depleted, the more robust cleptoparasitic larva manages to win over the agaonid, resulting in its death. This competition for food, however, leads to the partial larval starvation of the torymid/pteromalid. The quantity of the food left over at the disposal of the developing pteromalid/torymid larvae in the different ovaries of a given syconium can vary depending on the duration for which the agaonid larva in each case has managed to survive. This leads to the production of variously sized torymid/pteromalid larvae. The size of the adults depends directly upon the size of the larvae, which itself is determined by the size of the cells of the larvae (Grosch 1948). This trophic factor, therefore, has a direct bearing upon the unisexual variations and unisexual polymorphism met with in the populations of certain species of torymid and pteromalid fig wasps.

This unisexual polymorphism is more commonly met with among the males and rarely among the females. In parasitic Hymenoptera, as shown by various authors, there occurs a differential mortality of the sexes as a result of partial larval starvation. Genieys (1925) showed in the case of *Habrobracon brevicornis* that partial larval starvation is less unfavourable to the male sex than to the female sex. According to Grosch (1948), it is haploidy rather than masculinity that enables animals to survive better under conditions of partial larval starvation. The female larvae which suffered from starvation would be eliminated while the males survive as dwarf individuals. This is probably why in cleptoparasitic fig wasps, dwarfism is rare among the females, while it is commonly met with in males.

Joseph (1959b) described the torymid species *Sycobiomorpha bimasculinum*, having two types of males: the alate and the apterous. The presence of these two types of males is advantageous to the species. It is suggested (Joseph 1984) that the existence of both apterous and alate males assures the fertilization of the maximum number of females by the apterous males inside the cavity of the syconium; those females that escape outside unfertilized, are fertilized by the alate males which await them on the surface of the fig wall. The importance of making observations on several aspects of biology including the mating behaviour of the fig wasps is therefore obvious and has great relevance to our understanding of the systematics of the group.

3.2 *Host specificity*

In general, the wasp pollinators (Agaonidae) do appear to be specific to their host fig, but exceptions do occur (Wiebes 1986). The best known of such exceptions is that of

Ceratosolen galili which is cleptoparasitic on *Ceratosolen arabicus* which lives in symbiosis with *Ficus sycomorus* (Galil and Eisikowitch 1968, 1969). However, *C. galili* does not pollinate its host plant. Michaloud *et al* (1985) found that two species of agaonids pollinate *Ficus ottoniifolia* in Gabon. The normal pollinator of *F. ottoniifolia* in localities from Zambia and Kenya in the south-west, to Gabon and the Cameroons more to the north-west, is *Courtella camerunensis*. However, only in Gabon, a second species, *Courtella gabonensis*, also pollinates the flowers of *F. ottoniifolia*. Berg (unpublished results) distinguished 5 subspecies of *F. ottoniifolia*, 3 of which are pollinated by *C. camerunensis*, a fourth by *C. gabonensis* and the fifth by *C. scobinifera*.

In the other families containing fig wasps (Torymidae, Pteromalidae and Eurytomidae) though the present state of our knowledge of the systematics does not permit a generalization, it may be stated that host specificity is generally found. It may be noted that related species of *Ficus* harbour related species of fig chalcidoids. The total fig wasp fauna of a Rhodesian fig, *F. thonningii* including 3 species of *Agaonidae*, appeared to consist 28 different species (Boucek *et al* 1981). Evidently more research on the insufficiently known fig wasps, is needed to throw more light on these and other exceptions to host specificity.

3.3 *Pollination*

It was Ramirez (1969) in Costa Rica and Galil and Eisikowitch (1969) in Israel, who almost simultaneously showed the presence of specialised structures and behaviours involved in pollination by fig wasps. Two kinds of containers for pollen transfer were subsequently recognised: mesosternal 'pollen pockets' and coxal 'corbiculae' on the fore legs. Ramirez (1978) envisaged the evolution of pre-agaonid wasps visiting the pre-*Ficus* inflorescence (to feed on pollen), by natural selection favouring the development of those forms with more setae on the mesosternum and coxae, in addition to those carrying pollen in the digestive tract. He distinguished 4 main systems of pollen transport viz (i) enhanced capacity to carry pollen in the buccal cavity or other hidden areas of the body (e.g. *Blastophaga psenes*); (ii) hairiness of the mesosternum (*Phagoblastus* sp.); (iii) corbiculae on front coxae (*Dolichoris* sp.); and (iv) mesosternal pollen pockets, with a transformation series, having the mesosternum with setae only, or with incipient pollen pockets, or with enlarged ones, or with almost closed pockets of the type Galil and Switzer-Pasternak (1970) described for *Platyscapa quadraticeps*.

Ramirez (1981) produced a cladogram for *Ficus* as well as for the agaonids. In general, it is possible to conclude that there is 'good fit' between the cladograms for the agaonid wasps and their *Ficus* hosts, in that most sections of *Ficus* have their own genus of pollinating wasps. On a higher level this need not work, as there are many other factors that influence the course of the phylogeny in agaonids.

3.4 *Morphological features*

Fig wasps exhibit wonderful adaptations for life inside the fig syconia. Pronounced sexual dimorphism is common as perhaps the most important of their adaptations. With few exceptions, the males are vermiform, apterous and live only for a short

duration of a few hours during which time they accomplish their sole functions of locating and fertilizing their females. Their strongly chitinized integument, greatly developed prothorax, well developed anterior and posterior legs and small middle legs, their apterism, vermiform shape and peculiar telescopic arrangement of the 7th, 8th and 9th abdominal segments (in agaonids), are all adaptations for facilitating these functions. A detailed analysis of their morphological features and transformations in different groups of agaonids are discussed by Wiebes (1982a,b). The important morphological features pertaining to the females of agaonids are given below.

3.4a *Shape of the head*: The dorso-ventrally depressed and distinctly longer head, facilitates entry through a tubular ostiolar opening of the syconium, as found in the figs of sections *Galoglychia*, *Malvanthera* and *Pharmacosycea*.

3.4b *Structure of antenna*: Normally, the scape is short and robust, but in some genera it is more or less lengthened (e.g. *Agaon*, *Alfonsiella*, *Allotriozoon*, *Elizabethiella*, *Paragaon*, *Pleistodontes*, *Nigeriella*, *Tetrapus*). The long scape may be used as levers pushing apart the bracts while entering the syconia through the ostiole. Presumably the short scapes are also used in the same way.

In most genera, the pedicel is distinctly longer than wide and bears a number of backwardly directed conspicuous spines. As far as the shape and conformation of the third segment is concerned, there appears to exist a transformation series, from its simple nature without a pronounced apical elongation (e.g. *Alfonsiella*) to a segment that is produced more apically (e.g. *Agaon*), in which the elongation forms a distinct appendage. The appendage may be secondarily segmented as in *Ceratosolen*.

3.4c *Reduction of mouth parts*: The most primitive state of reduction is the one in which the maxilla consists of a distinct galea and stipes and bears a palpus, and also the labium has a palpus (e.g. *Tetrapus*). At the end of the process of reduction, the labium and maxilla are reduced to one composite structure in which the labium may be unrecognisable (e.g. *Liporrhopalum*).

The appendage of the mandible may be completely fused with the body of the latter and bear ventral lamellae (as in the *Blastophaginae*), or it may be truly appended to the mandible and bear ventral rows of teeth (as in the *Agaoninae*).

3.4d *Length of the ovipositor*: Ramirez (1974) believed that a long ovipositor is a primitive character. He also related the change from monoecious figs as hosts, to dioecious figs, with a change from long to short ovipositors, and finally back to long ovipositors when the figs revert to monoecious state.

In the case of males, the following characters are relevant.

3.4e *Reduction of the antenna*: The most complete antenna has 7 or 8 segments, viz the scape, pedicel, 3 funicular segments and a club formed of 2 or 3 parts (e.g. *Platyscapa awekei*) but usually the number is smaller, i.e. only 4 in some *Blastophaga* spp.

3.4f *Antennal torulae*: A shifting of the toruli seems to have occurred in two steps: viz their approximation towards each other in the mid line, and their restriction in

separate pockets in the head capsule. Widely separated toruli are found in *Alfonsiella*, *Nigeriella*, *Platyscapa* etc while in *Blastophaga*, *Kradibia*, *Liporrhopalum* etc they are approximated and can be pulled back into pockets. This condition is even better developed in *Ceratosolen*, where the scrobes may be open, half closed or closed over almost the whole length.

3.4g *Reduction of some mouth parts*: As in female, the labium and maxillae are greatly reduced or lost. However, distinct labium and maxillae are present in most of the species of *Ceratosolen*, *Dolichoris*, *Pegoscapus*, *Pleistodontes* and *Tetrapus*. In some there are remnants of maxillo-labial complex (e.g. *Alfonsiella*, *Allotriozoon* and *Kradibia*), while in certain others they are much reduced or totally absent (e.g. *Agaon*, *Dielagaon*, *Liporrhopalum*, *Eupristina* etc).

3.4h *Tarsal segments*: The primitive condition is that all tarsi have 5 segments. In several genera there is a reduction in the number of tarsal segments of the fore, mid and hind legs.

3.4i *Genital armature*: In some genera, the male genitalia carry digiti, with or without claws, as in a few *Blastophaga*, most *Ceratosolen*, *Dolichoris*, *Kradibia*, some *Liporrhopalum*, 2 species of *Platyscapa*, most *Pleistodontes* and *Tetrapus*. The digiti are usually absent in other groups.

3.5 *Behavioural aspects*

The males of all fig chalcidoids are protandrous. On emergence they gnaw a hole in the wall of the fig ovary in which they developed and emerge into the cavity of the syconium. Now they start their search for, and unerringly locate the particular fig ovaries enclosing their respective females. This ability of the males to locate the ovaries containing their own females without any trial and error is reasonably supposed to be based on the chemoreception and the detection of specific chemicals (pheromones) produced by the females, by the sensillae located on the tip of the antennae of the male concerned (Joseph 1958). The protandrous males also make exit holes in the wall of the fig ovaries enclosing their teneral females. This act is carried out, irrespective of whether copulation takes place with the female while it is still enclosed in the fig ovary as in Agaonidae or after it has emerged into the syconial cavity as in the torymid (*Apocryptini*) and pteromalid (*Philotrypesini*) fig wasps, or outside the ostiolar opening on the external surface of the fig syconium as in the pteromalid (*Epichrysomallini*) fig wasp, *Odontofroggatia galili* and most likely in the eurytomid fig wasps. It has also been found that at least in the case of *Philotrypesis*, the male helps the females to go out of their gall flowers by slightly pulling them out by means of the mandibles and then allowing them to come out on their own.

In the case of the *Agaonidae*, the male cuts out a hole on the wall of the fig ovary enclosing the female and introduces its tubular abdomen to copulate with the teneral female inside. In the torymid *A. bakeri* (Abdurahiman and Joseph 1978a,b) and the pteromalid *Philotrypesis caricae* (Lichtenstein 1919; Grandi 1921; Joseph 1958), copulation occurs soon after the females emerge from their gall ovaries. However, in the pteromalid *O. galili*, the males tunnel through their syconial wall, close to the ostiolar area, by means of their large, sickle-shaped mandibles, generally making a

circular hole (0·4 mm dia), through which they crawl out. Several such males assemble on the top of their syconia, running excitedly here and there around the ostiole; they even push each other. As the first female emerges through the ostiole, one of the males grasps her firmly with his legs and mounts her from above. The female now runs about carrying the amatory male rider; the male curls his abdomen underneath the female's gaster and copulation follows.

4. Discussion

4.1 *Classification*

So far the main criterion adopted for classification of fig wasps has been an analysis of the morphological characters, supplemented by some biological evidence wherever available. Such analysis seems to suggest that at least some of the relevant groups have nothing to do with the family *Torymidae* under which most of the fig wasps (other than the agaonids) had been classified (Boucek *et al* 1981). The Agaonidae form a distinctly separate family. It seems that the subfamily *Sycoecinae* which is yet unplaced, is related to the *Agaonidae*, probably as a less specialised (plesiomorphic) group, pointing to their common origin.

The *Agaonidae* is divided into two subfamilies—the *Agaoninae* and the *Blastophaginae*. On the basis of the characters discussed above, some groupings become apparent, though the characters applied in such groupings must be used with caution (Wiebes 1982b). The tripartition of the *Agaoninae* has been made mainly based on zoogeographical arguments. The subfamily *Blastophaginae* show an accumulation of presumably primitive character-states in some genera, each of which may be quite recognisable, but only with difficulty, united with the others. The classification of the families *Agaonidae* and *Ficus*, in certain aspects, run almost parallel, i.e. at the level of fig sections. On a higher level this poses problems. It seems inevitable that the classification of the *Agaonidae* needs to be refined, in order to clear up the many unresolved relationships. It is, therefore, essential that more and more aspects of the biology of these insects are involved and treated in detail so that the analysis is based more on secure foundations.

In the family *Torymidae*, the subfamilies *Toryminae* and *Sycophaginae* undoubtedly have had closer links. The *Sycoryctinae* constitutes another torymid subfamily—but this view is rather questionable, as there are some reasons to classify them under the *Pteromalidae* as well (Boucek *et al* 1981). The subfamilies *Otitesellinae* and *Epichrysomallinae*, closely related to each other, could well be placed under the *Pteromalidae*. However, the *Epichrysomallinae* seem to be closely related to the eurytomids. The remaining groups of fig wasps, viz *Eurytomidae* and *Ormyrinae* do not pose many problems. Among these, the ormyrids show very close affinity with the Torymidae and may, therefore, be regarded even as a subfamily of *Torymidae*.

In the light of the above discussion, a brief classification of the fig chalcidoides (modified from that given by Boucek *et al* 1981) emerges. Based on the present state of our knowledge of the biosystematics of the fig wasps, an attempt has been made to place most of the various genera under the categories of tribes, subfamilies and families as given in table 1.

Table 1. Placement of the genera of fig wasps (superfamily *Chalcidoidea*) under the categories of tribes, subfamilies and families.

Genera	Tribe	Subfamily	Family
Tetrapus, Agaon, Nigeriella, Pleistodontes, Allotriozoon, Elizabethiella, Alfonsiella	—	Agaoninae	Agaonidae
Blastophaga, Eupristina, Waterstoniella, Platyscapa, Dolichoris, Kradibia, Ceratosolen, Maniella, Deilagaon, Liporrhopalum, Pegoscapus	—	Blastophaginae	
Crossogaster, Phagoblastus	—	Sycoecinae	(unplaced, Boucek *et al* 1981)
Physothorax	—	Toryminae	
Sycophaga, Eukoebelea, Parakoebelea, Idarnes	Sycophagini	Sycophaginae	Torymidae
Apocrypta	Apocryptini		
Ormyrus	—	Ormyrinae	Torymidae or Pteromalidae
Philotrypesis	Philotrypesini		
Sycoscapter, Arachonia, Sycoryctes, Sycoscapteridea, Sycorycteridea, Watshamiella	Sycoryctini	Sycoryctinae	Pteromalidae
Otitesella, Micranisa, Walkerella, Grandiana, Grasseiana	—	Otitesellinae	Pteromalidae
Sycobia, Pembertonia, Sycophilodes, Sycophilomorpha, Sycotetra, Camarothorax	—	Epichrysomallinae	Pteromalidae
Eurytoma, Syceurytoma, Ficomila, Sycophila	—	Eurytominae	Eurytomidae

5. Conclusion

In the systematics of fig wasps, we are confronted with two basic problems. Firstly, there exists a number of genera and subgenera the definitions of which are extremely vague and limits of variation little known. Secondly, in many cases the host *Ficus* spp. occur as well defined and genetically distinct varieties, each species of *Ficus* having its own specific agaonid pollinator. Yet another problem is that of the occurrence of considerable convergence in various characters of the fig wasps, presumably brought about from the similarities of the microhabitats of the wasps involved. These characters may be very successful as specific determinators but are often of limited value as generic or tribal characters. In the males, they include the reduction of the mid leg, oligomery of tarsal segments, fusion of thoracic terga, loss

or reduction of eyes and reduction of mouth parts. In the females, elongation of the head, lengthening of the mandibular appendage, obsolescence of venation, loss of tibial spurs and reduction of mouth parts are some of them (Hill 1967). Such similarities in the morphological modifications of the fig wasps are obviously correlated with similarities observed in their biology, ecology and behaviour.

The taxonomy of only about 100 species of fig wasps have been worked out, though about 900 species of *Ficus* (which are mostly tropical in distribution) are known; the biology and pollination ethology of only about 10 species of wasps have been studied so far. Also very little work has been undertaken to elucidate the morphological aspects of fig wasps using the scanning electron microscope (Joseph and Abdurahiman 1984). The combined efforts of entomologists and botanists of different parts of the world aimed at in-depth studies on the agaonid—*Ficus* symbiosis, will enable us to understand the phylogeny and co-evolution of the different sections of the genus *Ficus* and of the different associated genera and species of fig wasps.

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Biosystematics of Chalcididae (Chalcidoidea: Hymenoptera)

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Abstract. The Chalcididae represent a large group of parasitic Hymenoptera which parasitise pupal or larval stages of various insects including several pests. Their phylogeny is not so far clearly known, but a Eurytomid—Torymid line of accent could be postulated. There is a general resemblance in their adult behaviour such as emergence, courtship, mating, oviposition, feeding etc. Their hosts belong to Lepidoptera, Diptera, Hymenoptera, Neuroptera, Coleoptera and Strepsiptera.

Keywords. Chalcididae; Biosystematics.

1. Introduction

The Chalcididae (S. Str.) represents a large group of parasitic wasps (Hymenoptera: Chalcidoidea) which parasitise various insects, many of which are of economic importance. Their hosts include the blackheaded caterpillar of coconut, the cotton leaf-roller, the padyskipper, the diamond back moth of cabbage, the gypsy moth, the castor capsule borer as well as extremely large number of other pests. Unfortunately many species of Chalcididae look very much alike while they differ widely in habits. Hence, precise identification of the species or infraspecific categories is highly important in any host-parasite studies involving these insects which are important, interesting and difficult (taxonomically) parasitic insects.

The study of Chalcididae may be said to have begun well before 200 years ago when Linnaeus (1758) discovered and reported a few species. Since then several authors have contributed to the knowledge of this family and some of the important contributions are those by Fabricius (1775, 1787), Walker (1834, 1841, 1862), Westwood (1829), Dalla Torre (1898), Dalman (1820), Spinola (1811), Motschulsky (1863), Forster (1859), Fonscolombe (1840), Cresson (1872), Klug (1834) and Kirby (1883). Since, the monumental work of the classification of Chalcidoidea by Ashmead (1904) our knowledge of the family has been greatly enhanced by the studies of Cameron (1897, 1906), Crawford (1910), Schmitz (1946), Waterston (1922), Girault (1915), Gahan (1938), Gahan and Fagan (1923), Ruschka (1922) and Masi (1929). During recent years contributions to our knowledge has been made by Boucek (1952, 1982), Burks (1940, 1960), Grissell and Schauff (1981), Steffan (1951, 1961), Erdos (1955), Habu (1960, 1962), Nikolskaya (1952), Mani (1938) and Narendran (1975, 1984).

While most of the above contributions were mainly on the taxonomy of these Chalcids, the following authors also studied many aspects of their biology. Some of the important contributions are of Dufour (1841), Parker (1923), Roberts (1933), Howard and Fiske (1911), Burgess and Crossman (1929), Dowden (1935), Paillot (1923), Faure (1926), Kamal (1938), Hanna (1934), Cherian and Basheer (1938), Schneider (1939, 1940), Garthwaite (1939), Taylor (1943), Haroonkhan and Verma (1946), Jayaratnam (1941), Clausen (1940), Steffan (1961), Sychevskaya (1966), Roy *et*

al (1940), Narendran (1975), Joy (1976), Leonard (1971), Simser and Coppel (1980) and Thompson (1983).

2. Phylogeny and systematics

The family Chalcididae is perhaps the oldest of all other Chalcidoid families. They have evolved probably from the same ancestral stem from which the Torymids and Eurytomids evolved. Among the members of the family Chalcididae it is difficult to construct a phylogenetic tree in the absence of a clear knowledge of the various important characters that played significant roles in the evolution of these families. It is thus necessary to arrive at some conclusion regarding the plesiomorphic and apomorphic features before attempting to interpret phylogeny of the subfamilies of Chalcididae and such an attempt is beyond the scope of this paper. At present we do not have conclusive points to postulate any theory or cladistic analysis to show the possible lines through which evolution has progressed among the various subfamilies of Chalcididae. At the same time students of this group should remember that every existing subfamily has developed specializations of its own and different features have evolved at different rates.

There are more than 1500 valid species and more than 150 valid genera of Chalcididae in the world. They are found in all continents but more number of their representatives are found in the tropical regions. The family Chalcididae is divided into 6 subfamilies (Narendran 1986): Chalcidinae, Brachymerinae, Dirhininae, Epitraninae, Haltichellinae and Smicromorphinae. Extreme morphological variations occur within many species and genera of this family and for the same reasons several species and genera were redescribed by several workers under new names. This is the main reason in studying this family of Chalcids.

3. Emergence

The adult emerges from the host through an exit hole made with the mandibles. After emergence the adults rests for a while and then starts to groom itself with its legs. If it is disturbed the adult may jump or run or fly readily.

4. Feeding

Adults take sugary fluids like honey, nectar etc, and occasionally feeds on the body juices of their hosts. Thus the adults can be observed during the morning hours in bright sunshine on the extra-floral nectaries of several plants. However, an exception to this can be met in the case of most species of the genera *Epitranus* and *Smicromorpha* which are found to be mostly nocturnal in habits.

The females are synovigenic in Chalcididae and hence they require a source of protein for their continuous production of eggs throughout their effective adult life. These needs of protein may be supplied by feeding on honeydew or plant nectaries (both of which contain free aminoacids), and body juices of hosts. Stinging with ovipositor is not always accompanied by oviposition. Females like the fluid exuding from its victim's body at the site of the sting. Such feeding ensures adequate quanti-

ties of proteins in addition to the carbohydrates derived from the feeding of the naturally occurring sugary fluids thus favouring the normal metabolism of the individual. Feeding of honey alone is insufficient and feeding of host's fluid is necessary to obtain enough protein needed for ovigenesis. However, our experiments with some species of Chalcididae showed that sugar containing nourishment such as honey prolonged the life of the adults, whereas the albumen nourishment (protein) out of host pupae showed no life prolonging effect.

5. Reproduction

The Chalcidids are biparental. Mated females can lay both fertilized (diploid) and unfertilized (haploid) eggs, which develop into females and males respectively. Unmate females produce only unfertilized (haploid) eggs, which develop into males (arrhenotokous parthenogenesis). Consequently, as in other Hymenoptera, the female can produce both female and male progeny during her life time.

6. Courtship and mating

A qualitative and quantitative analysis of courtship and mating behaviour shows some remarkable variations among genera species. However, the courtship and mating behaviour is dependent upon the inter-relationship of releasor and response in an innate behaviour pattern. One of the simpler, basic patterns was observed in several species of *Brachymeria*. The sequence of events is as follows: Male head nod-antennal contact-copulation. A more advanced pattern is exhibited by several species of Haltichellines (species of e.g. *Antrocephalus*, *Hockeria*, *Invreia*, *Psilochalcis* etc). This pattern includes the following phases: Male head nod-antennal contact-mount-postmount antennation-backward movement of male-copulation-dismount. The post-mount antennation is certainly an advanced trait since the same is met with in some of the advanced families such as Eulophidae, Aphelinidae etc. The development of long antennae and consequent development of broad and deep scrobe must be regarded as advanced characters developed along with this type of advanced courtship behaviour.

7. Host selection and oviposition

The major stages of host selection by Chalcidids are (i) habitat selection, (ii) host finding, (iii) host acceptance and (iv) host suitability. Our knowledge of habitat selection is very limited. While several species of genera *Brachymeria*, *Antrocephalus*, *Haltichella*, *Hockeria*, *Nearretocera*, *Invreia* etc, are met with near flowers of plants like *Cassia tora*, *Pisum sativum*, different types of grasses etc, a few such as *Megachalcis*, *Trigonura*, *Notaspidium*, *Chalcis*, *Bucekia* etc, are rarely encountered. The Epitranines were seen mostly in woody plants in dry weather while *Smicromorpha* is very rarely found in localities near their host *Oecophylla smaragdina* Fabr.

The host finding behaviour of a few species studied shows that it is mainly depended upon chance-encounter and chance rules until the parasite was within a certain distance near the host. A random search on the surface of the host plant is the basic

feature of host finding and when the parasite reaches within the critical distance-range (this varies between the hosts and the parasite species depending on several factors such as effect of kairomones, chemoreception etc) the parasite quickly finds its host either by chemoreception or by visual stimuli or by the combined action of both these factors.

Host acceptance in Chalcididae involves mainly the use of antennae and ovipositor. The mouth parts and tarsi hardly played any significant role in the species so far studied. The female generally examines the potential host with a drumming of the antennae over its peripheral surface. The duration of this exploratory phase may vary even among species of the same genus.

Host suitability is frequently evaluated by ovipositor. The ovipositor of many species have sensillae with which the host suitability could be tested.

The oviposition process was more or less similar in most species studied though a little variation occurred in the position of the tarsi while ovipositing. In *Brachymeria* the whole hind legs are used to hold on the host pupa firmly while in *Kriechbaumerella*, *Hockeria* and in *Antrocephalus* the tarsal segments mainly hold the hosts.

8. Taxes

The adults of several species studied by us are positively phototactic (we have not studied the nocturnal species of Epitranus and Smicromorpha). In confinement they have been found preferring to remain in lighted areas. They were very active when placed in bright sunlight or electric light. The male's mating drive could be reinforced through the direct effect of the sunlight or electric light.

9. Death feigning behaviour

Some species of *Brachymeria* were found showing a death feigning behaviour as seen in several groups of beetles and other insects. When disturbed the adults were found dropping down from their resting places freezing and remaining still as though dead for about 10–20 s.

10. Fecundity

Chalcidid females are synovigenic. Eggs are produced in two ovaries, each composed of 3 ovarioles. They develop and mature continuously throughout the life span but after a short preoviposition period of 1–3 days. Data about the actual fecundity of Chalcidids are rather scarce. However, the reproductive capacity of most species of Chalcididae known is low. This is because they have only 6 ovarioles and eggs are relatively large and each ovariole can hold one ovariole at a time. Hence the maximum number of eggs laid in a day by a single female varied from 6–8.

11. Longevity

The longevity of adults depends on several factors such as availability and nature of food, weather conditions etc. Without food the adults survived for only 2–4 days. With adequate nutrition and between thermal thresholds, longevity is longer at lower

temperatures. In *Brachymeria lasus* at a constant temperature of 9–12°C females fed with honey (50%) lived for 32–130 days with a mean of 65·1 days.

12. Larval behaviour

There are 5 larval instars. They have well developed respiratory system with branching and ramified trachea. The circulatory, digestive and nervous systems are simple. The larval duration mostly lasts for 4–10 days and the pupal duration of about equal length. A prepupal stage is also met with, the size and shape of the mandibles and diameter of thoracic spiracles help determine the stage of the larva.

13. Seasonal history

This mainly depended upon local climatic conditions. In Southern India many species were found throughout the year. Their population however, dwindled during extreme summer and in extreme monsoon period. Their population was at its maximum during the postmonsoon period in the case of several species.

14. Hosts

The majority of the hosts of Chalcididae belong to Lepidoptera. A few species are parasitic secondarily on Dipteran and Hymenopterous parasites. Dipterous maggots or pupae are often parasitised by some Chalcidids as primary hosts. Sarcophagids, Calliphorids and Muscid mature maggots or pupae are attacked by some species of the genera, *Brachymeria*, *Dirhinus* and *Hockeria*. Tripetidae forms host of a few species of *Dirhinus*. A few species of *Chalcis* parasitise Stratiomyidae. Syrphidae is parasitised by a species of *Spilochalcis* (*S. hirtifemora* (Ashmead)).

Besides Ichneumonidae and Braconidae some other Hymenopterous species of Agridae, Elasmidae and Megachilidae are parasitised by the species of *Spilochalcis*, *Hockeria* and *Neochalcis* respectively.

Some species of *Trigonura*, *Brachymeria*, *Spilochalcis*, *Proconura* and *Phasganophora* are parasitic on Coleoptera. A few species of the genera *Lasiochalcidia* and *Hybothorax* are parasitic on Neuroptera. Strepsiptera forms host of some species of *Hockeria*.

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Biosystematics of rice brown planthopper and rice green leafhoppers

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Abstract. The Brown planthopper, *Nilaparvata lugens* (Stal) is causing serious damage to rice cultivation in tropical countries of Asia for the last 10–15 years. Green leafhoppers, *Nephotettix* spp. are also assuming serious proportions in different parts. This has been attributed to improved rice production technology, especially with the introduction of high yielding varieties. Damage is not only through loss of sap as a result of feeding but also by transmitting diseases in this process. Various reasons have been attributed like breaking of resistance, migration, insecticidal treatment etc. One such reason, which led to the biosystematic studies of these pests is the assumption that this is due to evolution of new forms referred to as biotypes. Of late biotypes have also been reported in *Nephotettix virescens*. This term is basically used with reference to differential ability of the insect to infest particular rice cultivars having resistant genes. This phenomena is attributed by some scientists, not to the breaking of resistance, but to the evolution of new forms which are different from the original population. As per the conventional taxonomy, this new form is similar to the original form, and hence biosystematists attempted various methods like chemical and honeydew analyses, cytology, acoustic behaviour, morphometrics, etc in order to help the breeders in differentiating and identifying these and also to find out the mechanism and causes evolving such biotypes.

Keywords. BPH, GLH biosystematics.

1. Rice brown planthopper

The rice brown planthopper (BPH) belongs to family Delphacidae of Homoptera which can easily be distinguished from all other families of the suborder by the presence of mobile spur at apex of post tibia. The genus *Nilaparvata* can be distinguished from other genera of the family by the presence of one or more lateral spines on the hind basitarsus. So far 14 species (Mochida and Okada 1979) are recorded throughout the world, out of which only *N. lugens* has been recorded as a pest which causes serious damages to rice cultivation both by sucking sap from the plant and also by transmitting plant diseases like grassy stunt and ragged stunt. Species is characterised by its typical male genitalia, especially styles and aedeagus and by lateral lobes of female ovipositor.

BPH is widely distributed from Pakistan to Japan and many islands of Southeast Asia (Claridges 1985). BPH existed in most of these countries since ancient times referred under different synonyms (Paik 1977). Since early part of this century frequent outbreaks of this pest have been reported from some of these countries and from early seventies situation became quite alarming. Dyck and Thomas (1979) in a conservative estimate reported US $ 300 million loss in paddy due to BPH and grassy stunt disease transmitted by it.

Intensive research on all possible aspects of BPH is in progress so that suitable pest management strategy could be evolved to combat this menace. Until recently resistant rice varieties and insecticides were satisfactory means of controlling these insects. But soon it was found that varieties resistant in one country were susceptible

in another. Also the varieties resistant earlier became susceptible after sometime in the same country. This was attributed to various factors like breaking of resistance, overcoming of resistance, migration, insecticidal treatments or to the existence or evolution of different biotypes in different areas. This term biotype in planthoppers and leafhoppers though initially was used with reference to differential ability of the insect to infest particular rice cultivar having resistant genes, various other methods were attempted to find out the differences which could be used to distinguish and identify these and also to see if these could provide some clue to evolution of such biotypes. Such studies include acoustic behaviour, mate choice experiments, honeydew analysis, morphometry etc.

1.1 *Honeydew studies*

As already stated biotypes have been recognised based on the differential virulence to the resistant varieties. Virulence is taken as ability of the insect to infest and damage the plant and damage is correlated with insect feeding. Further, amount of feeding, weight gained and quantity of honeydew excreted are found to be highly correlated. This, therefore, led to honeydew studies to find out its utility in identification or confirmation of the biotypes.

Initially honeydew studies at IRRI (1978) were taken up to determine its value in measuring levels of resistance. Both quantitative and qualitative analyses were done. Former by measuring ninhydrin stained areas of honeydew on filter paper and also by measuring volume of honeydew. Latter by densitometer measurements of honeydew colour intensity and by measurements of colour intensity of amino acids separated through paper chromatography.

The filter paper technique for measuring honeydew excretion was found helpful in identifying biotypes. TN 1 which has no resistance gene and, therefore, is susceptible to all biotypes has larger areas in all the 3 biotypes and shows no significant differences. Whereas Mudgo which is resistant to biotype 3 but susceptible to biotype 2 shows significant difference between the areas of two biotypes; biotype 2 having larger area than the biotype 3. Similarly ASD 7 which is resistant to biotype 2 but susceptible to biotype 3, has larger area in case of biotype 3 and significantly smaller area in biotype 2.

Similarly, study of honeydew volume at IRRI also showed distinct differences in honeydew excretion by both biotypes feeding on susceptible and resistant varieties. Biotype 3 feeding on IR 40 excreted significantly more honeydew than those feeding on IR 42.

But according to Sogawa (1977), a biotype of BPH excretes as much honeydew even on certain resistant varieties as it does on susceptible ones and suggested that gustatory blockage of feeding is the principal cause of varietal resistance of rice to this insect. At the same time intraspecific variation in gustatory responses in BPH is considered to be a possible reason for occurrence of the biotypes which can feed on resistant varieties.

Both honeydew areas on filter paper and honeydew volume techniques are practical methods but need readily available materials and could be used by scientists anywhere for determining feeding activity which in turn can reflect biotypic nature of population, if any.

Tests with colour intensity of honeydew which was measured with densitometer after treating it with ninhydrin for amino acids or aniline hydrochloride for sugars show some promise for detecting and differentiating biotypes. The peak was higher in biotype 3 on ASD 7 to which it is susceptible whereas peak for biotype 2 is much lower to which ASD 7 is resistant. Similarly on Mudgo, peak for biotype 2 is higher to which it is susceptible while for biotype 3 is much lower to which it is resistant.

Further study of honeydew by measuring the colour intensity of amino acids of honeydew, which are separated through paper chromatography holds promise for identification of biotypes by using standard differential varieties. Works at IRRI (1978) showed that susceptible varieties have more number of amino acids with high colour intensity than the resistant varieties.

However, honeydew studies by Claridge and Hollander (1980) on 3 inbred biotype cultures of *N. lugens* from Philippines found wide range of variation within each biotype and large overlap between them. Means for each biotype on 3 test cultivars—TN 1, Mudgo and ASD 7 were significantly different from each other but means for these 3 biotypes on the same cultivar were not significantly different amongst themselves. Each biotype population had individuals which could be attributed to other biotypes. Honeydew studies conducted on randomly collected field samples showed that these are mixtures of individuals which could be attributed to biotypes 1, 2 and 3. Based on these findings showing variability and overlap of biotype populations they felt the use of biotype in this insect is misleading. Besides, their studies on morphology, cytology, biochemistry and acoustic behaviour of these Philippine biotypes did not show any significant differences. They concluded that sympatric biotypes of Philippines are simple genetic variants and therefore, suggested that use of term biotype masks the inherent variability of the population.

1.2 *Acoustic behaviour*

In forties, Ossiannilsson's (1949) pioneering work on sound production in some Auchenorrhyncha showed that besides loud and audible sounds produced by Cicadas, smaller Auchenorrhyncha like leafhoppers and planthoppers also produce species-specific sounds which are faint and inaudible. Since then, especially during the last decade extensive research was carried out to find out all aspects of these sounds such as mechanisms, structures involved in production and perception, nature of sound, its role in behaviour, speciation, etc. Use of tape recorders and electronic equipments for sound analysis have facilitated this line of research enormously. Since the most distinctive sounds are produced in behavioural context of pair formation most of the studies have been conducted on this aspect.

According to Ichikawa (1977) females of *N. lugens* have no tymbal or special sound producing organ and sound production is accompanied by obvious dorso-ventral vibration of the abdomen. In males 1st and 2nd abdominal terga are specialised as tymbal organs. He stated that the sound produced though faint was within audio-frequency range, but the planthopper never responded in mating behaviour even from 1 to 2 cm distance. Various other experiments conducted by him, indicate that the stimuli involved in the mating behaviour were neither auditory nor visual nor olfactory. It was concluded that planthoppers transmit vibratory signals directly to solid substrate on which they lived and that they perceived these signals by some vibration receptors. And therefore, he felt that physical properties of the substrate

were likely to affect manner of transmission. According to Claridge (1985) abdominal vibration normally does not result in contact with the substrate. The vibrations are transferred to the plant through the legs and perhaps sometimes also through the feeding stylets which may be inserted during calling. According to him any of the variety of internal chordotonal sensilla in different parts of body might serve as vibration receptors.

According to Ichikawa (1977) and Claridge (1985) both sexes of *N. lugens* produce characteristic signals with distinctive pattern and grouping of pulses. Female calls are generally simpler than those of males of the same species and often consists of regularly repeated pulses. Sexually mature male and often also female call spontaneously on their food plants. Responsive insects reply by emitting their own signals and acoustic exchanges usually end with a male making contact with female.

Studies by Claridge *et al* (1987) on calling sounds of male and female of *N. lugens* on two different hosts in Philippines show that the female call on rice consists of sequences of regularly repeated pulses, whereas from weed grass, *Leersia hexandra* it produces similar calls but of less than a third pulse repitition frequency (PRF) of rice plant. This PRF was found very important for conspecific responses. They observed that these planthoppers associated with two different hosts i.e. rice and *L. hexandra*, both males and females responded to the recorded calls of the opposite sex of their own host population very significantly, more than they did to those of the other. However hybrids could be obtained in no choice experiment. Based on these PRF differences Claridge *et al* (1985) have taken them as two closely related sibling species with no constant morphological difference between them.

However, in view of Ichikawa's (1977) observation on the effect of physical properties of the substrate on transmission, it is not clear if calls were recorded on each other's hosts and if any differences were found.

The same team of workers (Claridge *et al* 1985) while studying the geographical variations in male calls of *N. lugens* showed that populations from Solomon Islands differed significantly in mean PRF from the populations of Australia. This difference was thought to be responsible for behavioural incompatibilities observed during hybridization experiments between these two populations. A series of crosses were made between the two populations and male calls from successful crosses were recorded. PRF of these calls were significantly nearer to male calls of the other population and lower or higher than the mean of their own populations. This again shows the importance of PRF in communication.

Role of acoustic signals of planthoppers and leafhoppers in speciation was argued by Claridge (1985) based on acoustic studies of *N. lugens*. For evolution of species, according to most of the scientists isolation mechanism is a must. Acoustic signals being species-specific and since they could be transmitted only through substrate lead in premating isolation both call-wise as well as host-wise. His work as already explained earlier showed that morphologically sibling species from the same geographical areas but having different hosts have significantly different PRF means. And the hybrids produced between these, under no choice experiment had intermediate PRF which shows the hereditory nature of the character.

Claridge *et al* (1985) also studied the PRF of *N. lugens* from widely scattered localities under standard conditions. Each was characterised by particular range of variations, some significantly different from others e.g. Australian population. Hybridization is difficult in populations that differed most in male PRFs. And

herefore, calling sounds help in maintaining geographical isolations. But whenever ıybrids are obtained between such divergent populations they are completely ıormal, fertile, produce F1 and F2 generations with no obvious anomalies. From his he concluded that though they are different in call characteristic, genetically they ıre very closely related and differentiation of calls occurs in advance of genetic differentiation.

.3 *Mate choice experiments*

Mate choice experiments conducted by Claridge and Hollander (1980) on 3 biotypes of Philippines also gave the same results as honeydew tests. They were found to be variants of one freely interbreeding biological species. They were not clearly separable and different from each other. They felt that since in *N. lugens* inheritance of virulence was of polygenic nature, field populations normally varied in virulence and evolved in response to a particular rice cultivars in a particular area and therefore, same biotype occurring in widely distributed areas was not possible.

1.4 *Morphology*

Biotypes of *N. lugens* were found to be identical morphologically. However, the idea of differentiating biotypes through numerical taxonomy with morphometrical accuracy was considered possible by Okada (1977). Accordingly Sogawa (1978) studied quantitative morphological variations but found that except for the significant variations in frequency distribution of the number of spines on the hind basitarsus, Philippine biotypes show no significant differences.

Similar morphometrical studies were conducted by Bhattacharyya *et al* (1983) on macropterous forms of Pantnagar and Hyderabad populations. Little microtypic numerical variations recorded on some characters were unable to separate these two populations morphologically.

2. Rice green leafhoppers

Nephotettix virescens (Distant) and *N. nigropictus* (Stal) belong to the family Cicadellidae of the order Hemiptera, whose members can be distinguished by elongate hindlegs and tibiae having rows of spines all along the length. The genus can be distinguished from other genera by its characteristic green and black colour markings, shape of head and male genitalia. Both the species are found all over India and are widely distributed in other parts of Southeast Asia. Both nymphs and adults cause damage by sucking the sap from leaves and stems. Besides, they cause serious damage indirectly by transmitting the causal agents of various rice diseases. Out of these Tungro (a virus disease) and yellow dwarf (a mycoplasma disease) occur in India.

2.1 *Nephotettix spp.—Biotype*

Recently biotypes have been reported in *Nephotettix* species based on their differential infestation and differential tungro transmission ability to various rice

cultivars at different places. Karim and Pathak (1979) have reported Bangladesh biotype and Philippines biotypes of *N. virescens* based on the resistant genes in the rice cultivars. Ling *et al* (1981) tested 10 rice varieties at 4 centres, Rajendranagar and Cuttack in India, one in Philippines and another in Indonesia for possible biotypes of *N. virescens* based on life-span and Tungro transmission ability and reported that rice varieties Ambemohar 159 at Rajendranagar and Pankhari 203 at Cuttack could be used as differentials for biotypes. Thus problem of biotypes in *Nephotettix* is at an early stage and populations from different places are likely to be taken as biotypes in future depending on their characteristics.

Ramakrishnan (1983) studied morphometrics of various populations of *N. virescens* and *N. nigropictus* collected from different agroclimatic zones to find out whether such studies can be used for differentiating these populations. These studies showed that the morphometry could be used to distinguish these populations and it was suggested that such studies should be extended to the already designated biotype populations. In biotypes, besides varying agroclimatic factors rice cultivars are also likely to play their role on the size of insect depending on susceptibility or resistance and therefore, such studies should be more useful in distinguishing and detecting the existence of biotype populations. While 16 characters were chosen for study, minimum and the most important of these had to be selected after attempting such studies on some more populations and by applying suitable statistical analysis.

Yusof (1982) studied calling sounds of *N. virescens* and *N. nigropictus* populations from different parts of Asia which showed variations. This shows the utility of these calls in biotype studies of these species.

Studies on honeydew in green leafhopper (GLH) conducted at IRRI (1978) were aimed at finding out different resistance levels in IR rice cultivars and since the results showed differences in densitometer readings of honeydew, they also show promise in biotype studies.

2.2 *Nephotettix spp.—Hybrids*

In *Nephotettix* species, besides biotypes there is another problem. Both *virescens* and *nigropictus* are widely distributed and are sympatric species. Though they are usually clearly separable on the basis of colour patterns, in fields sometimes morphologically intermediate individuals are found. Hybrids of these two species were produced in the laboratory which showed intermediate morphological characters (Ling 1968; Inoue 1983).

2.2a *Hybrids: Morphology*: Ramakrishnan and Ghauri (1978) studied male genitalic characters of spines on pygofer and aedeagus in the field collected individuals showing intermediate colour markings. None of these specimens had the combination that is usual in either of the species. This has led to the assumption that such specimens are hybrids between *virescens* and *nigropictus* resulting from crosses in nature.

Subsequently Ramakrishnan (1983) studied these spine characters in two populations of *N. virescens* and 3 populations of *N. nigropictus* collected from widely separated geographical areas. Specimens only with clearcut colour markings and other external characters typical of *virescens* and *nigropictus* were selected for the

purpose. No specimen showing intermediate form or colour markings was included for the observations. In spite of such careful selection of specimens for studies, much variation was observed in the number of spines on aedeagus and pygofer. It was, therefore, concluded that variations in these characters are natural, intraspecific and not because of hybridization. Study of these characters in the pure bred material were suggested for the further verification of this observation.

Accordingly in 1986, aedeagal spines were studied in two samples of pure bred material of *N. virescens*. The spines in one sample varied from 2–3 to 5–5, while in another these varied from 3–4 to 5–5 whereas parents in both the cases had only 4–4. This confirms the earlier observations that spines variation on the aedeagus in *Nephotettix* species is intraspecific and not due to hybridization.

2.2b *Hybrids*: *Acoustic calls*: The above morphological observations have been further corroborated by various works on calling sounds of these species.

Acoustic sounds in GLH were first demonstrated by Ichikawa (1976, 1979) from the abdominal vibration in both male and female and he clarified that these are essential stimuli to each other before copulation.

Inoue (1983) studied acoustic signals of 4 species of *Nephotettix* which included *virescens* and *nigropictus*. Both male and female calling sounds showed that they were species-specific. He observed no communication between male and female of two different species and found that by their species-specific nature were concerned in maintenance of reproductive isolation among species. He found no identical or confusingly similar sound wave among these species studied.

Inoue also tried number of interspecific crosses and tested hybrids from successful crosses for morphological and acoustic properties. Hybrid call study was aimed at finding out the effectiveness in discriminating the mating partner. Response between the hybrid male and female derived from the same cross was clear but between the hybrid and their parental species they displayed various responses being from poor to clear.

Hybrid calls though distinct from parent calls were not distinctive of hybrids. They were irregular order of connections of elements from parents.

Yusof (1982) studied calling sounds of males and females of both the species *virescens* and *nigropictus* and their hybrids produced in the laboratory and also of the intermediate individuals collected from fields. Both male and female calls show clear differences between the two species. Calls of F1 hybrids were very variable and different from either parents though most individual hybrids showed some recognizable elements from the calls of both the parental species.

Calls of field collected intermediates were found to be either of *N. virescens* or of *N. nigropictus* type but not of hybrids. This again strengthens the view that hybridisation does not occur in nature.

Most of the works mentioned here were an attempt in identifying and differentiating biotypes as defined by breeders. Some did show promise in this direction, and showed differences in different biotypes while others gave contradictory results. This may be due to evolution of new form from the originally declared biotype populations. Because confirmatory results were obtained in 1977–78, while contradictory results came from 1980 specimens. It is likely that the so called biotype populations have undergone changes by then. Thus though these various methods like acoustic calls, honeydew analyses, mate choice experiments and

morphometrics are complementary to each other and might help in differentiating the already designated biotypes or identifying the new ones, these findings will be of help only for a short time, the biotype being a changing phenomenon. However, all these various methods mentioned here can be utilized by using comparable standard techniques for finding out any difference in widely scattered geographical populations so that breeders could anticipate differential response to the varieties to be released and therefore, can adopt the screening and breeding methods accordingly.

From the perusal of literature on BPH and GLH for more than a decade one may conclude that we have landed once again on the starting point as regards definition and differentiation of the biotypes and the problem to be tackled. Despite these studies which show some differences but could not provide well defined separable characters, the biotype has to be defined on the basis of differential response of the insect to particular rice cultivar having resistant genes. Further, from the results obtained so far, it appears that the term biotype would have to be restricted to the localities i.e. local populations showing susceptibility to the rice cultivars which have been declared as resistant to BPH or GLH in other parts of the world. Reasons in such cases, for the differential virulence can only be agroecosystem in the area. But when the same resistant variety becomes susceptible in the same area in course of time it could mostly be a case of overcoming resistance and/or adaptability. And, therefore, to face this problem continuous screening and breeding of resistant varieties in well defined areas having similar agroecosystem seems to be the only answer.

The term biotype is not inappropriate as it is based on behaviour aspect. But it would be better if the biotype is referred along with the locality, period (year) and plant cultivar, instead of with numbers. For example, Biotype Kerala, 1965, Mudgo—meaning Kerala biotype population, which showed susceptibility to Mudgo in 1965.

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Biosystematics of gall aphids (Aphididae, Homoptera) of western Himalaya, India

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Abstract. Garhwal and Kumaun ranges of Himalaya include 250 odd species of aphids infesting a number of agricultural and forest plants. Out of these only 52 species (20%) are found to be gall formers. Majority of these gall aphids (63%) are indigenous. Except 4 species which produce true galls on stems, all other species are known to produce leaf fold or leaf roll or leaf base galls.

In general, the gall forming aphid species are heteroecious i.e. alternate between their primary and secondary hosts in different periods of the year. However, a few have been found to be autoecious i.e. monophagous. These aphids are also highly polymorphic in nature. Unless morphs from both of their primary and secondary hosts are available their identities in some cases are difficult. It has been observed that the morphology of aphid galls, karyomorphology, host association, life cycle pattern and natural enemies (both predators and parasites) help in the identity of such aphids more precisely, particularly in the closely related species complexes.

Keywords. Gall aphids; gall formation; gall morphology; Life cycle; host association; cytogenetics; predators; parasites; Western Himalaya.

1. Introduction

Aphids or plant lice, a homopteran insect group occupy a special status among the plant pests particularly due to their special mode of reproduction. They damage the plants directly through sucking up of the plant sap and there by inflicting several symptoms on the plant surface of which plant galls are very important. Among the indirect damages the role of aphids as vectors of several plant viral diseases received world wide importance (Eastop 1977).

Aphids although are predominant in the temperate regions (Dixon 1984) have a world wide distribution except Greenideinae and Hormaphidinae that make up about 7% of the world fauna and now restricted to South East Asia and America. A total of 3742 species of aphids are known (Eastop and Hille Ris Lambers 1976) from the world. From the list of Agarwala and Ghosh (1984) and also from further accounts, published during 1982 to 1986, a total of 691 species are now known from India which constitutes at least 15% of world fauna.

Available records show the preponderance of aphid fauna in the mountains of India, particularly in the Himalayan regions. Several papers relating to distribution of aphids in different regions of India (Agarwala 1986; Agarwala and Ghosh 1985; Chakrabarti 1983; Ghosh 1983, 1986; Ghosh and Raychaudhuri 1980; Maity *et al* 1983; Saha and Raychaudhuri 1986) also emphasised the host-aphid relationships. Aphids, one of the major gall forming insect groups, however, received very little attention in the Indian context.

of Western or Kumaun-Garhwal Himalaya. This part of Himalaya lying between the Central or Nepal Himalaya and North west or Punjab Himalaya received special attention for aphidological studies in recent times. To avoid duplications of the recent review on the biology of gall aphids (Wool 1984) discussions were restricted on the species or related species found in the area. However, for general understanding mention of a few species not found in the area could not have been avoided.

2. Fauna of the area

Through the works of Chakrabarti (1972), Maity (1979), Saha (1986) and also through several stray reports about 250 odd species are known from the area. This includes several species infesting agricultural and forest plants where they sometimes inflict specific damage symptoms in the form of galls.

2.1 *Taxonomy of gall aphids*

The association of aphids with true galls on host plants is considered a primitive one. Among the 52 gall forming species known from the area (table 1) of the superfamily Aphidoidea, all belong to the family Aphididae. The other 2 families viz Adelgidae and Phylloxeridae although known by gall forming species in other places, are not represented in the area. Galls of Aphididae belong to 3 subfamilies viz Pemphiginae, Aphidinae and Hormaphidinae.

Taxonomy of gall forming species is very much complicated for several reasons viz complex life history, extreme morphological variations in different polymorphic forms, different host associations etc. Knowledge of taxonomy of these aphids remain incomplete unless and until several phenomena like host association, gall morphology, life cycle and biology in general are worked out and employed along with morphology of aphids for their identification. Besides, some other tools like karyomorphology, biochemical studies (isoenzymes, gall biochemistry etc), natural enemies also may further help in their identification. The state of our such knowledge on gall forming aphids of western Himalaya are also highlighted.

3. Gall formation and gall aphid

It is very difficult to define a gall particularly an aphid gall. A gall is formed due to interaction between the offensive stimuli involving growth substances released by insects and the defensive response by plants (Rosenthal and Janzen 1979). Although the precise mechanism of gall formation is not yet known, but plant growth hormone IAA (either transmitted with the saliva or formed in the plant by oxidative transamination of tryptophan by saliva enzymes) and free amino acid in the saliva is suspected for cecidogenesis (Miles 1968, 1972). The specific response of the plant is the result of an altered metabolism due to the action of an alien chemical stimulus together with the wounding effects as a result of feeding or oviposition inducing alternation in the cellular and metabolic environment initially around the feeding area. Different kinds of tissue reorganisation including the major cecidogenic phenomena like hypertrophy and hyperplasia ensue leading to gall formation (Ananthakrishnan 1984). Gall formation is a complex phenomena involving the

recanalisation and reorientation of plant development and such growth activities result in the insects becoming partially or completely enclosed, so that gall insects grow, mature and reproduce within the galls (Ananthakrishnan 1984). Insect galls have limited neoplasm and thus continue to develop only so long as the cecidogenia is associated or its immediate metabolic products are present in the tissue (Mani 1973). The responsibility of gall initiation in aphids lies with the fundatrices. In Pemphiginae single fundatrix may initiate a gall. The first instar nymph of fundatrix having stout fore legs in *Epipemphigus niisimae* (Aoki and Makino 1982) initiates gall on the leaves of poplars in Japan. During such activities, other first instar nymphs and/or later stage of nymphs may invade the partially formed galls when a strong resistance and fighting between the gall inhabitant and the intruding individuals are noticed. In western Himalaya similar phenomenon was observed in *Epipemphigus imaicus* while the first instars of this species were initiating galls on the leaves of *Populus ciliata* (Chakrabarti S, unpublished results). Feeding of several nymphs may sometimes be required for the development of galls in *Salvum werlheime* (Wertheim and Linder 1961) and in *Mordwilkoja vagabunda* (Ignoffo and Granovsky 1961). Two different types of galls on *Pistacia atlantica* are formed by the fundatrices and the apterous offsprings of some Fordini (Koach and Wool 1977; Wertheim 1954) and in *Thecabius populimonalis* (Maxson and Knowlton 1929). Similarly, coloured caterpillar galls and leaf fold galls are produced by *Eumyzus prunicolus* in western Himalaya.

Though the tendency to produce disorganised growth and malformation in plants is common, true galls are always positive, directional responses resulting in disharmonic growth effects with the polarity related to the insect rather than the rest of the plants (Ananthakrishnan 1978).

The classification of aphid galls is rather conflicting. Many species cause leaf deformation in a definite pattern on specific host and sometimes all the individuals of the colony can not be covered within such deformations. In the literature these structures are referred to as leaf galls, leaf roll galls, pouch galls, curl galls etc and more commonly as pseudogalls. The other forms which completely cover gall forming individuals are true galls. Here, both these types have been taken into consideration.

Only 12 pemphigine species form true galls on stem, leaves and petioles and the remaining species under this subfamily and all other species under subfamilies Aphidinae and Hormaphidinae form leaf roll, leaf fold, leaf curl galls etc.

4. Endemism

Quite a large number of aphids are endemic. Except *Epipemphigus* which is also found in Pakistan, no other gall aphid genus is truly endemic. But as many as 24 species (46%) are endemic.

5. Life cycle

Wool (1984) discussed the available information on the life cycle of gall aphids. These informations do not contain any biological peculiarities of many species occurring in India, particularly in western Himalaya.

The pattern of life cycle is unique and highly adapted for individual species with a view to reproduce successfully, to tide over some adverse conditions and to avail of the maximum chances of security for survival. Gall aphids are in general host alternating (heteroecious) and represented by both parthenogenetic and sexual cycles (holocyclic). This general or primitive pattern is still maintained in several species. Chakrabarti *et al* (1985) provided some accounts of the life cycle pattern of Pemphigine species infesting poplar in western Himalaya. The typical heteroecious holocycle in Pemphiginae has been found modified in several species of the genera *Baizongia*, *Forda*, *Geoica*, *Pemphigus* and *Thecabius*. Perhaps the typical condition is maintained in the species of *Epipemphigus*, *Eriosoma*, *Kaltenbachiella*, *Prociphilus* and *Tetraneura*.

Several adaptive strategies are found in the species of *Pemphigus* in the area. Apterous viviparous generation is missing (suppressed) in *Pemphigus dorocola*, *P. matsumurai* and *P. mordvilkoi*. Ghosh (1984) following Maity and Chakrabarti (1981) wrongly mentioned the presence of apterous viviparous generation in *P. mordvilkoi*. However, this generation is always present in *P. siphunculatus*. At least 3 species of *Pemphigus* viz *dorocola*, *mordvilkoi* and *siphunculatus* lead autoecious life on poplars (figure 1). Fundatrices in these 3 species have a long life and are found till the end of autumn and the sexuparae are produced within the galls and can be located there till the advent of winter. At least in *mordvilkoi*, sexuparae hibernate in winter and sexuals are laid in early spring (Chakrabarti S and Mandal A K, unpublished results). The gall forming generations on *Pistacia integerrima* by the species of *Baizongia*, *Forda* and *Geoica* of the tribe Fordini are of very short duration. These aphids lead a 2-year cycle (figure 2). After completing one full year and also the summer months of the second year on the secondary hosts, alate immigrants (sexuparae) are produced which migrate to *P. integerrima* for laying sexuals and ultimately hibernating eggs. The fundatrices are produced from these eggs in spring which initiate specific galls where they live upto first half of the summer months only then they again migrate to their secondary hosts (Hille Ris Lambers 1957; Wertheim 1954; Bodenheimer and Swirski 1957). There is also a possibility that some species partially adopted anholocyclic life as found in Australia, Central and Northern

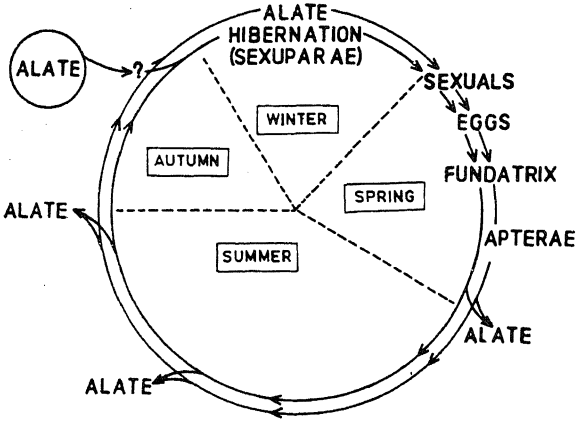

Figure 1. Autoecious life cycle pattern in some *Pemphigus* species in Western Himalaya.

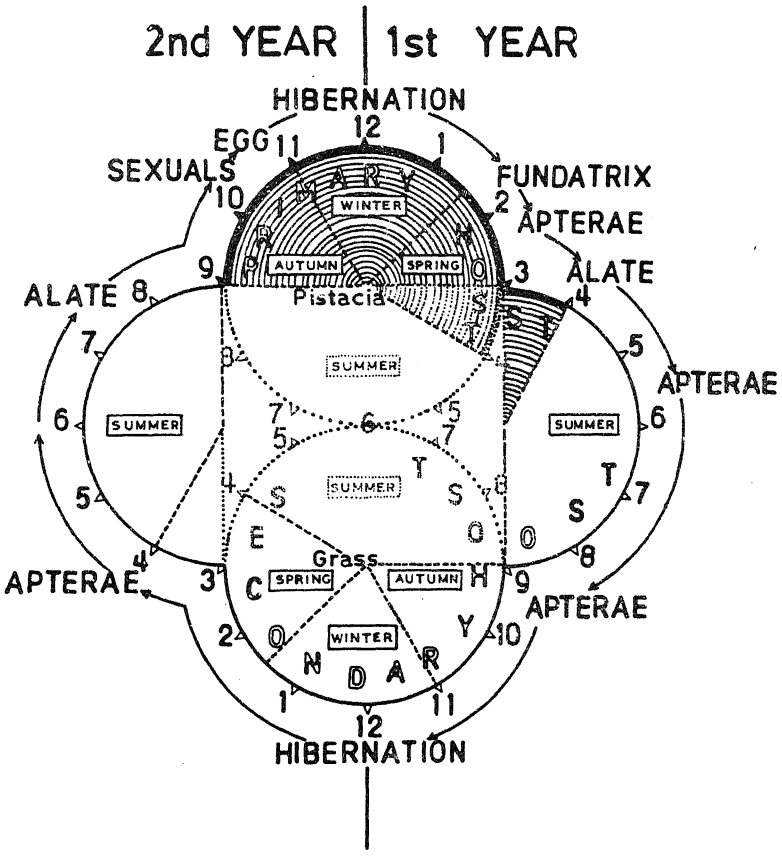

Figure 2. Two-year life cycle pattern of *Forda marginata* in western Himalaya.

Europe and in America (Hille Ris Lambers 1970). *Eriosoma* species except *E. lanigerum* alternate between species of elm (*Ulmus* spp.) and roots of Rosaceae particularly *Ribes* spp. The apple wooly aphid, *E. lanigerum* leads heteroecious holocyclic life on *Malus* spp., *Pyrus* spp. and *Ulmus* spp. in North America (Danielsson 1979; Smith 1985) and anholocyclic life on *Pyrus* spp. and *Malus* spp. in Israel and Denmark (Heie 1980) and an abortive gamic cycle at least in Switzerland. In western and north west Himalaya this species leads autoecious anholocyclic life on its secondary hosts, *Malus* and *Pyrus* spp. where it hibernates in roots and cracks and crevices of stem of the plants. In India this species sometimes produces sexuals (Fotedaer and Kapur 1943) which seem to be abortive. A similar condition is observed in *Thecabius affinis* which produces galls on *Rananculus hirtellus*, a secondary host in other countries (Mordvilko 1934; Tullgren 1909; Danielsson 1979). In western Himalaya *Geranium wallichianum* and *Geranium* spp. are the secondary hosts of this species where it lives on roots. This species has never been collected on poplar in India. Species of *Tetraneura* alternate between *Ulmus* spp. (primary hosts) and grasses (secondary hosts). However, the possibility of anholocyclic life like the species of Fordini viz *T. nigriabdominallis* and *T. radicicola* can not be over ruled since these species also occur even at very low altitudes where its primary host does not grow. *Prociphilus* spp. has

Lonicera, *Syringa* and *Cotoneaster* as their primary hosts. Only *P. himalayaensis* has been collected from its secondary host, *Pinus wallichiana* where the species lives on its roots. The morphological similarities demands the correlation of *P. taxus* described from the roots of *Taxus baccata* (secondary host) with the material received from *Syringa emodi* (primary hosts) through transfer experiments. *Kaltenbachiella pallida* is heteroecious but details of its life cycle is not known.

Among the Hormaphidine species *Hamamelistes miyabei* produces leaf galls on *Betula alnoides* and *Betula utilis*. Species under this genus in other countries alternate between *Betula* and *Hamameleis*. *H. spinosus* produces galls on flower buds, hibernate on birch for a longer period and cannot complete the entire cycle within one year (Ghosh 1985; Pergande 1901). In western Himalaya, *H. miyabei* was found on *Betula* till June and further information on its life cycle is lacking. In general, members of Nipponaphidini produce galls on its primary host *Distylium*, *Hormaphidini* on *Hamamaleis* and Ceratiphidini on *Styrax* (Sorin 1966). Several changes in the life cycle pattern leading to anholocyclic monophagism either on its primary host or on its secondary host have been found in this group. Several species of the genera *Astegopteryx*, *Cerataphis*, *Ceratovacunna*, *Chaitoregma*, *Heminipponaphis*, *Indoregma*, *Neothoracaphis*, *Pseudothoracaphis* and *Tuberoaphis* lead autoecious anholocyclic life on their secondary hosts in the area.

In subfamily Aphidinae, gall formers are mainly restricted to two genera viz *Eumyzus* and *Myzus* where 10 species produce galls. Other important gall producing genera are *Aphis*, *Avicennina*, *Brachycaudus*, *Cryptaphis* and *Dysaphis*. All these species are heteroecious and possibly holocyclic. Alate males are produced on secondary hosts in several species. Number of generations, both on primary and secondary hosts are variable even in closely related species of *Myzus*. Many of these species have several secondary hosts and some of them may be designated as summer reservoir. Peach leaf curling aphid, *Brachycaudus helichrysi* has atleast 20 secondary hosts.

6. Host aphid association

6.1 *Host specificity*

Gall forming aphids are impressively host specific on their primary host. Each family or subfamily or tribe is usually restricted on single plant family or genus. Secondary hosts of many groups may however, also be specific or at least belong to the same plant family. The host association and host alternation is more specific and rigid in Pemphiginae than in any other groups. In Aphidinae, *Prunus* spp. serves as the primary host of several species of *Avicennina*, *Brachycaudus*, *Eumyzus* and *Myzus*.

The polyphagy particularly in Pemphiginae in many cases, turns out to be the result of morphological similarity of genetically different strains or races of aphids adapted to morphologically similar but genetically distinct races of host plants (Wool 1984). It has been found that in Pakistan, *Populus niger* and *P. euramericana*, a hybrid from the cross of European *P. nigra* and American *P. deltoides*, growing side by side but only the former plant is heavily infested by *Pemphigus lichtensteini*, while the latter remains uninfested (Habib and Ghani 1970). A similar hybrid developed from *P. ciliata* and now growing in and around Joshimath also shows resistance to

Epipemphigus imaicus and *Pemphigus mordvilkoi* infestation. During late autumn or early winter sexuparae were found to settle in the cracks and crevices of these hybrid plants but fail to initiate galls on them in the next spring. But the nearby wild plants of *P. ciliata* are always nearly infested. A similar case was also found in America (Grigarick and Lange 1968). Whether a plant will or will not be successfully colonised by aphids may depend on physiological, nutritional, ecological or genetical factors of both aphids and host plants.

6.2 *New host acquisition*

A few new host acquisitions have also been found in the area. *Pistacia* is the primary host of Fordini where these aphids produce galls. But at an altitude below 100 m *Pistacia* does not grow and *Forda orientalis* accepts *Toona ciliata* (Meliaceae) as its primary host where it produces leaf marginal galls. *T. affinis* produces leaf galls on *Rananculus hirtellus* instead of *Populus.* The former is the secondary host of this species. *Geranium* spp. serve as the secondary host of this species in western Himalaya.

6.3 *Gall morphology*

The shape and size of aphid gall in general are extremely variable (Ghosh *et al* 1981; Bhattacharya 1982). Inspite of such diversities, gall of a particular species is very specific and helpful for identifying especially some pemphigid species which are very laborious to separate morphotaxonomically (Chakrabarti S and Mandal A K, unpublished results). *Eriosoma kashmiricum* and *Eriosoma* sp. are hardly separable by morphological characters but very distinct in gall morphology. In comparison to pemphigids, other galls are less specific in their morphology (table 1). Colour of galls, particularly in *E. imaicus, Eumyzus pruni, E. prunicolus, Forda marginata, Geoica urticularia* and *Pemphigus matsumurai* are quite distinct. Conical galls, characteristic of *Tetraneura* species on *Ulmus* spp. are more in number on individual leaf in *T. (Indotetraneura) javensis* but a few per leaf in other *Tetraneura* species. Other leaf gall characteristics of different genera are globular hard leaf base galls of *Kaltenbachiella pallida,* cystolith shaped leaf base galls of *P. matsumurai,* caterpillar galls of *E. imaicus,* globular basal galls of *G. urticularia.* Galls of *Baizongia pistaciae* on *Pistacia integerrima* are unique in having an elongate structure developed from leaf buds or axils. Leaf spiral galls of *Eriosoma* on *Ulmus* spp. and leaf fold galls of *Prociphilus* on *Lonicera* and *Syringa* are hardly separable by their structures but have some specificity with regard to the time of occurrence.

Galls of Aphidinae, when considered irrespective of their host plants are hardly separable. But on *Prunus,* species of several genera (table 1) are found but those of *Avicenenna indica, E. pruni* and *E. prunicolus* maintain their identities.

7. Cytogenetical studies

Cytogenetics of aphids have several fascinating chapters. But almost all works on gall aphids in India is restricted so far on the karyomorphology of parthenogenetic viviparous morphs. As many as 685 world aphid species are known (Blackman 1980,

Table 1. Gall aphids and their host plants, nature of galls and chromosome number in western Himalaya.

Aphid species	Host plant (primary)	Gall type	Chromosome number (2n=)
Subfamily: APHIDINAE			
Aphis clematidis	*Clematis buchaniana*	Leaf curl	
A. fabae	*Solanum nigrum*	Leaf curl	
A. gossypii	*Cuminis* sp.	Leaf curl	6, 8
Avicenenna indica	*Prunus cornuta*	Leaf fold	
Brachycaudus helichrysi	*Prunus amygdalus*	Leaf curl	11, 12
	P. domestica	Leaf curl	13
	P. persica	Leaf curl	
Ceruraphis eastopi	*Viburnum cotinifolium*	Leaf fold	
Cryptaphis garhwalensis	*Lamium album*	Leaf curl	
Eoessigia indica	*Cotoneaster obtusus*	Leaf curl	
Eumyzus eastopi	*Pyrus vestita*	Leaf roll	
E. hydrangi	*Hydrangea scandens*	Leaf fold	
E. impatiensae	*Impatiens* sp.	Leaf fold	
E. pruni	*Prunus cornuta*	Leaf marginal	
E. prunicolus	*P. cornuta*	Leaf fold and caterpillar	
Dysaphis sp.	*Cladeolus* sp.	Leaf fold	
Dysaphis sp.	*Pyrus vestita*	Leaf roll	
Hayhurstia atriplicis	*Chenopodium album*	Leaf fold	14
Hyalopterus pruni	*Prunus cornuta*	Leaf curl	10
Myzus cornutus	*Prunus cornuta*		
Myzus formosanus	*Polygonum capitatum*	Leaf roll	12
M. mumicola	*Prunus* sp.	Leaf roll	
M. ornatus	*Prunus* sp.	Leaf roll	12
M. persicae	*Callicarpa* sp.	Leaf fold	10, 11, 12, 13, 14
M. sorbi	*Sorberia tomentosa*	Leaf roll and leaf curl	12
Rhopalosiphoninus ehretis	*Schizandra grandiflora*	Leaf	
Sappaphis sp.	*Cotoneaster obtusus*	Leaf roll	
Tubicauda hydrange	*Hydrangea* sp.	Leaf roll	
Subfamily: HORMAPHIDINAE			
Hamamelistes miyabei	*Betula alnoides*	Leaf fold	
Tuberaphis loranthi	*Loranthus cordifolius*	Leaf roll	
Subfamily: PEMPHIGINAE			
Baizongia pistaciae	*Pistacia integerrima*	Elongate	24
Epipemphigus imaicus	*Populus ciliata*	Caterpillar	18
Eriosoma sp.	*Ulmus laveagata*	Leaf base	
Eriosoma kashmiricum	*Ulmus* sp.	Leaf spiral	12
E. lanigerum	*Pyrus malus*	Leaf fold	12
E. phaenax	*Ulmus montanus*	Leaf spiral	
E. ulmi	*Ulmus* sp.	Leaf spiral	12
Forda marginata	*Pistacia integerimma*	Leaf marginal	24, 25, 26, 27, 28, 32
F. ricciboni	*Pistacia integerrima*	Leaf marginal	18
F. orientalis	*Toona ciliata*	Leaf marginal	
Geoica urticularia	*Pistacia integerrima*	Globular leaf base	
Kaltenbachiella pallida	*Ulmus* sp.	Leaf base	28
Pemphigus dorocola	*Populus ciliata*	Stem gall	20
P. matsumurai	*P. ciliata*	Leaf base	12

Table 1. (*Contd.*)

Aphid species	Host plant (primary)	Gall type	Chromosome number (2n=)
T. mordvilkoi	*P. ciliata*	Stem gall	
T. siphunculatus	*P. ciliata*	Stem gall	
Prociphilus sp. A.	*Syringa emodi*	Leaf curl	10
Prociphilus sp. B.	*Lonicera quinculocularis*	Leaf fold	
Prociphilus sp. C.	*Cotoneaster* sp.	Leaf fold	
Prociphilus himalayensis	*Lonicera quinculocularis*	Leaf fold	
Tetraneura javensis	*Ulmus* sp.	Leaf conical	
T. nigriabdominalis	*Ulmus* sp.	Leaf conical	12, 14, 22, 24, 26
T. ulmi	*Ulmus* sp.	Leaf conical	
Thecabius affinis	*Ranunculus hirtellus*	Leaf fold	38

1986; Kuznetsova and Shaposnikov 1973; Kurl 1986). Chromosome number does not suggest an ancestral number for Aphididae (Blackman 1980).

In India karyotypic analysis has been made in some 80 odd species and chromosome formulae based on relative percentage length of individual chromosome pairs have been ascribed for some 35 species (Khuda-Bukhsh 1986). Variations in chromosome number and structural heterozygosity have been noticed. Chromosome number of only 21 gall forming species are known (table 1).

Since gall forming genera and species are almost always with a sexual phase, they represent more stable karyotype. In several genera differences in gross chromosome morphology can be useful to the taxonomists. Within species karyotype variation is relatively common in aphids and instances on structural heterozygosity are particularly numerous in species and groups which have partially or completely abandoned the sexual phase of thelotoky (Blackman 1980). Thus heterozygosity are found in *B. pistaceae*, *B. helichrysi*, *F. marginata*, *Myzus persicae*, where partial or complete anholocycly are found.

Karyotypic studies of closely related genera, species, host alternating species, polymorphic and polyphagous species in different geographical areas (clones) may provide some clue for better identification or rather confirmation of specific identification.

3. Natural enemies

Galls provide a natural protective environment to the individual insect. Adaptive strategies of natural enemies to get into the galls, particularly true galls are very important for their effectiveness. A number of predators and parasites are associated with the gall aphids. Normally the association of predators with the leaf fold and leaf roll galls are heterogeneous, while the parasitic complexes are more specific both in the true galls and pseudo-galls.

3.1 *Predators*

Major predatory complexes are coccinellids, anthocorids, syrphyids and neuropterans. As many as 16 coccinellid species are found (Ghosh D, Debnath N and Chakrabarti S, unpublished results). Only *Halyzia sanscrita*, *Harmonia eucharis*, *Harmonia* (*Leis*) *limidiata* and *Menochilus sexmaculatus* can predate on the true pemphigid gall

aphids. Individual leaf roll or fold gall aphid receive quite a large number of heterogeneous coccinellids and thus *E. lanigerum* have at least 11 coccinellid predators. *Coccinella setempunctata* and *H. eucharis* each have 7 gall aphid prey species while *Adalia tetraspilota* and *Oenopia sauzeti* have 6 and 5 prey aphids respectively. Hodek (1973) questioned the aphidophagous habit of *Platynaspis saundarsi*, which is found to predate *Eriosoma lanigerum* and *Sappaphis* sp. in the area. Similarly, *Halyzia sanscrita*, a mycophagous species exhibits aphidophagous nature in western Himalaya.

Anthocorids are more active and due to their small body size they can easily enter into the galls. At least 7 species are found with gall aphids (Ghosh D, Debnath N and Chakrabarti S, unpublished results). Only *Anthocoris confusus*, *A. flavipes*, *Orius niger* and *Ectemnus* sp. are found within the true galls. Another hemipteran predator, *Deraeocoris* sp. (Miridae) is associated with *Pemphigus mordvilkoi.*

Syrphyid larvae of 8 species (Ghosh D, Debnath N and Chakrabarti S, 1985) although very active on leaf fold or roll gall have not been found within the close galls except *E. imaicus* where *Episyrphus balteatus* and *Metasyrphus confrater* were recorded. These larvae were found only in the latter part of the gall life of aphids when the rim margin of the galls become loose and alate imigrants are ready for migration. The sluggish nature of syrphyid larvae and their large size generally prevents their entry into true galls. Aoki (1978) observed some syrphyid adults to lay eggs near the opening of the galls of *P. dorocola* so that the larvae immediately after their emergence can enter into the galls. No such incidence was observed in the area.

Neuropterans are less active on the gall aphids and only *Cunctochrysa jublngensis* larvae were found within the stem galls of *P. mordvilkoi.* Other predatory complexes are mites of the genera *Anystes*, *Podothombium*, *Allothrombium*, *Dinothrombium*, *Enemothrobium*, *Abrolophus*, *Balaustium*, *Paraerythaeus* sp. The chamaemyiid larvae *Leucopis* spp. are very common in both close and pseudogalls. An earwig, *Forficula beltzebula* was found within the stem galls of *P. mordvilkoi* and *P. dorocola* where the galls were completely cleaned. Whether this insect really predates on these aphids or takes only shelter has to be confirmed. Several spiders are also found with the gall aphids.

8.2 *Parasites*

Aphidiid parasites of 10 species under the genera *Aphidius*, *Diaeretiella*, *Ephedrus*, *Kashmiria* and *Trioxys* are found on gall aphids. Except *Ephedrus srinagarensis* and *Kashmiria aphidis* which are restricted on *Prociphilus* spp. all other species are polyphagous. No aphidiid parasite is found on the aphids producing true galls. Although aphidiid parasites try to avoid wax of the aphid colony (Stary 1970) *K. aphidis* is found to cover themselves with wax material present within the gall colony of *Prociphilus* spp. (Das and Chakrabarti 1986).

Several hymenopteran parasites of the family Encyrtidae, Eulophidae, Figitidae, Pheromatidae and Ichneumonidae are found to parasitise the syrphid predators of gall aphids. Hyperparasites of Aphidiid parasites under the family Cynipidae, Encyrtidae, Pteromalidae and Magaspilidae are quite common in the later part of parasitic infestation.

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Biosystematic studies on Agromyzidae from India

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Abstract. Pure systematics of Agromyzids attracted the attention of many entomologists quite early in the century, but investigations on their biosystematics is still to gain the necessary attention and recognition from entomologists. Their life history and feeding habits being so closely interrelated with the chemical nature of their host plant, investigations into their capability to select the right host plant become all the more important. It has become very essential to critically reassess their systematic position and the characters governing this sensitive interspecific relationship. Factors which require special attention in this regard are: (i) the organs and devices responsible for host recognition and host selection, (ii) correlation between the structure of these organs and the behaviour of these flies, (iii) recognition of special features in their biology and (iv) the study on the interrelationship between ecological factors and the special features in their biology.

Though agromyzid infestation causes damage to varied plant parts such as root, stem, leaf and seed etc there is striking structural similarity in the organs and devices responsible for host selection. This is mainly due to the fact that in all cases it is the adult fly which is responsible for choosing the host. As very little information is available on the correlations between structural adaptations for host recognition, selection and the behaviour of Agromyzids, an attempt has been made to highlight these aspects.

Keywords. Agromyzidae; generic composition; distribution; economic impact; parasitism.

1. Introduction

The Agromyzidae, popularly known as leaf miners, are one of the important Dipteran families of economic importance in the world. Studies on them during the past decade in the Gangetic plains show an increasing rate of infestation on quite a few important crop plants and a variety of ornamentals, very often reaching 100% plant infestation especially during the months of February and March.

The investigations in India on their systematics gathered momentum in the mid sixties whereas research on their ecology and biology started in the early seventies. A synopsis of oriental Agromyzidae by Spencer (1961) is perhaps the first attempt on their systematics in India followed by publication of a Monograph on the Agromyzidae from India by Singh and Ipe (1971). This publication includes descriptions of 97 species new to science. Sasakawa's Catalogue of Oriental Diptera records a total of 156 species from India without taking into account many of the accepted synonyms. As a family Agromyzids show a high degree of host specificity but unfortunately many of the records from India are without host linkage.

The present information on the group is in no way complete and many species still await exploration, and already known one, a more critical appraisal. An attempt has been made here to analyse their faunal composition, factors influencing their distribution along with the salient features of their life history, and interspecific relationship viz parasites etc. The biosystematic studies of Agromyzids from India will definitely be deficient in many ways with only limited information being available on

them. This presentation, therefore, should be considered only in the light of the fact that faunistic explorations of Diptera on the whole from India is far from complete.

2. Generic composition and seasonal variations

A total of 140 species belonging to 20 genera, viz *Agromyza* Fallen, *Japanagromyza* Sasakawa, *Melanagromyza* Hendel, *Hexomyza* Enderlein, *Tropicomyia* Spencer, *Epidermomyia* Ipe, *Ophiomyia* Brashnikov, *Phytobia* Lioy, *Cerodontha* Rondani, *Calycomyza* Hendel, *Amauromyza* Hendel, *Liriomyza* Mik., *Phytoliriomyza* Hendel, *Phytagromyza* Hendel, *Paraphytomyza* Enderlein, *Pseudonapomyza* Hendel, *Napomyza* Westwood, *Indonapomyza* Singh and Ipe, *Chromatomyia* Hardy and *Phytomyza* Fallen are recorded from India so far. There is a profound influence of vegetation on their distributional pattern and definite ecoregions are recognisible on the basis of their distribution. Their population can be divided into two major groups viz (i) Genera with palaearctic affinities dominant during winter months, and (ii) genera with tropical (monsoon) affinities dominant during summer months.

There is a distinct overlapping of these two populations (figure 1) in the Gangetic plains during the successive seasons closely interrelated with the seasonal vegetation. Dominant summer genera are *Melanagromyza*, *Tropicomyza* and *Epidermomyia* while *Chromatomyia*, *Phytomyza* and *Liriomyza* dominate during the winter months. Major Agromyzid groups and faunal regions recognisible are:

(i) Agromyzids with palaearctic affinity (dominant during winter months):	Northern elevations of Himalayan belt, higher elevations of western ghats and the Gangetic plains in winter months.
(ii) Tropical forms (dominant during monsoon season):	Peninsular India, including western coastal belt, north eastern border lands and the Gangetic plain in summer months.

Genera with palaearctic affinities *Chromatomyia* and *Liriomyza* are totally absent during the summer months in the Gangetic plains but they dominate during winter

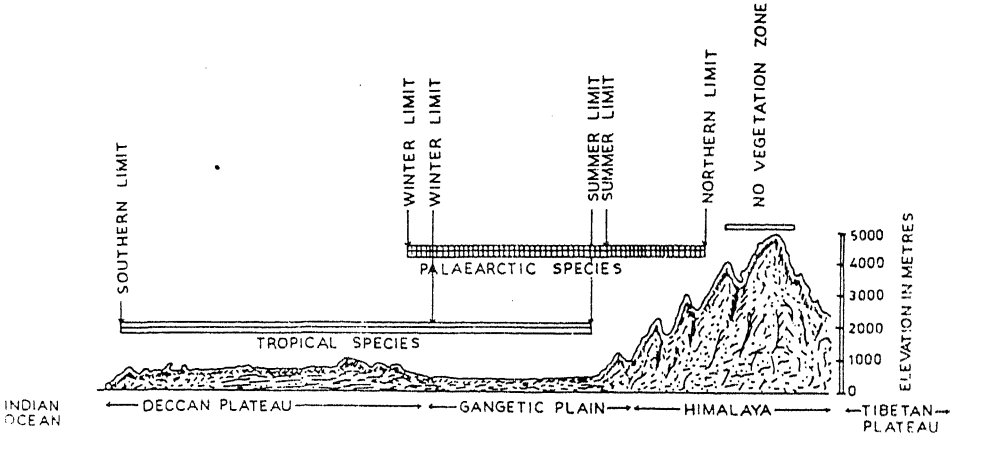

Figure 1. Summer and winter limits of palaearctic and tropical species.

months with infestation rate often reaching as high as 100% during February. However, it is interesting to note that the tropical genera *Melanagromyza* and *Epidermomyia* also manage to withstand the cold winter climate prevalent in the Gangetic plains to a considerable extent, the former reaching a maximum population level almost at the same time as that of *Chromatomyia*. However, their northern limits of distribution are very rarely over 240 m above MSL in the Himalayan foot hills (table 1).

Species like *M. obtusa*, *M. atomella*, *E. polyphaga*, *O. phaseoli*, *C. horticola* and *L. brassicae* are widespread in distribution (also seen extending into Rajasthan desert and Deccan plateau in winter months). Palaearctic genera like *Cerodontha*, *Liriomyza* and *Chromatomyia* were found in good numbers in the north eastern border lands. This region is climatically closer to the palaearctic region with lower temperature and higher humidity and hence the presence of these palaearctic genera in good numbers.

Two areas where palaearctic elements dominate (identified as rich in Agromyzids) are: (i) the Northern elevated Himalayan belt including north east India and (ii) the higher elevations of the western slopes of western ghats in the peninsular India.

Though both these areas are wide apart latitudinally they do have moderate to colder temperatures due to higher altitude and reasonably high humidity owing to comparative abundance of rain. The tropical plains as well as the Andaman and Nicobar Island chains are comparatively poor in variety of Agromyzids. This

Table 1. Distribution of Agromyzidae in Indian faunal limits.

Genera	Northern elevation	Gangetic plain	Rajasthan desert	Tropical west coast	Deccan plateau	North eastern border lands	Andaman and Nicobar Islands	Total
Agromyza	2	5		2	—	—	—	9
Japanagromyza	3	3		2	—	(2)	—	8
Melanagromyza	4	16	(2)	6	(2)	(1)	(2)*	26
Hexomyza	—	1		(?)	—	—	—	2
Tropicomyia	3	—		—	—	—	—	3
Epidermomyia	2	4	(2)	3	(2)	(1)	—	9
Ophiomyia	1	6		1	1(1)	—	—	9
Phytobia	1	1		1	—	1	—	4
Cerodontha	15	1		5	(1)	(2)	—	21
Calycomyia	1	—		—	—	—	—	1
Amauromyza	1	—		—	—	—	—	1
Liriomyza	8	3	(1)	1	(1)	(3)	—	12
Phytoliriomyza	4	—		1	—	1	—	6
Phytagromyza	3	—		3	—	—	—	6
Paraphytomyza	1	—		1	—	—	—	2
Pseudonapomyza	1	4		2	(1)	—	—	7
Napomyza	1	—		—	—	—	—	1
Indonapomyza	—	1		—	—	—	—	1
Chromatomyia	7	2	(1)	—	(1)	—	—	9
Phytomyza	1	—		1	—	—	—	2

Numbers in parentheses indicate overlapping species.
*Empty mines only.

conforms to the observations of Spencer (1973) about the poverty of Agromyzid species in the rain forests in East Africa, Ceylon, Philippines and New Guinea.

3. Altitudinal distribution

A study on their altitudinal distribution reveal that Genera *Cerodontha*, *Liriomyza*, *Phytoliriomyza* and *Chromatomyia* have emerged as cold loving genera with close affinities to their palaearctic counterparts. Majority of the known species are found at an elevation above 1000 m MSL (figure 2). In the case of *Phytoliriomyza* all the 6 known species are from areas above this elevation whereas in *Cerodontha* 18 out of the 21 species, in *Liriomyza* 6 out of 12 species and in *Chromatomyia* 6 out of 10 species are from above this elevation. *Melanagromyza* and *Ophiomyia* have got just one species respectively above this elevation and *Tropicomyia*, *Phytagromyza*, *Pseudonapomyza* and *Napomyza* have got none at all. The only *Melanagromyza* species is in all probability an introduced species.

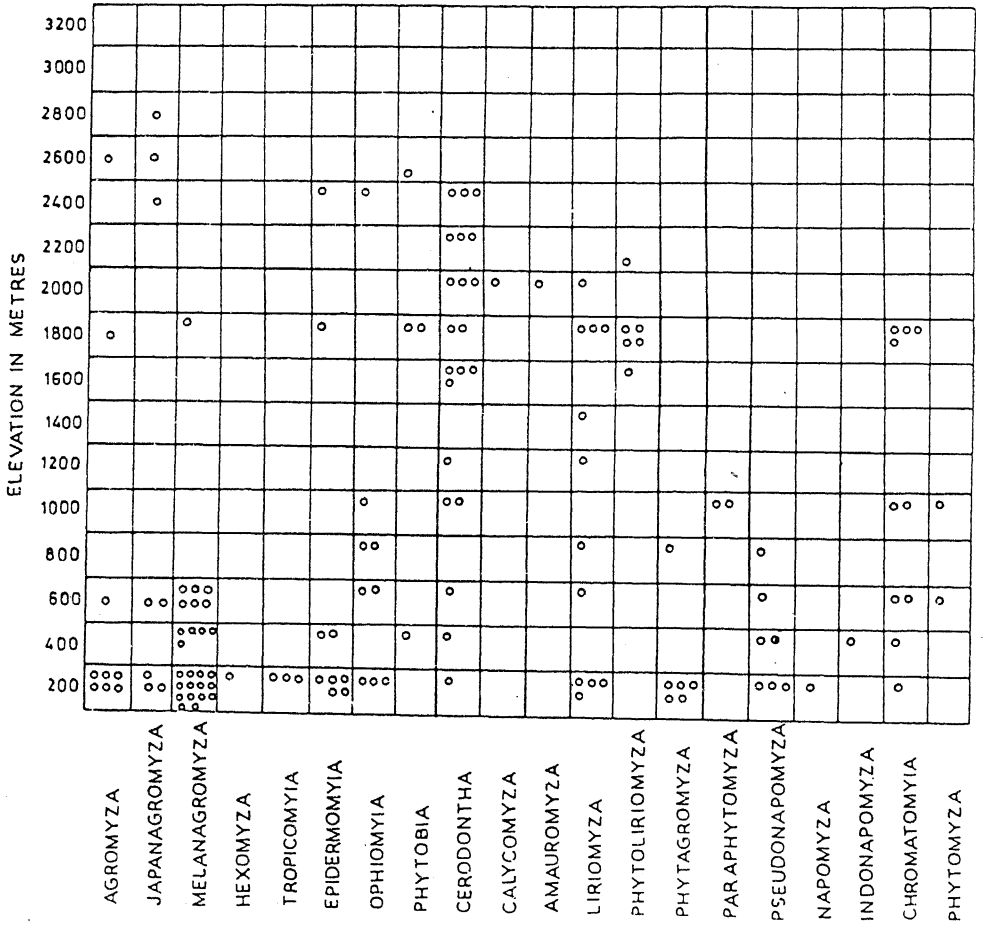

Figure 2. Altitudinal distribution of Agromyzids.

A few species that are widespread in distribution in the Indian faunal limits are *M. obtusa*, *C. horticola*, *L. brassicae*, *E. polyphaga* and *E. atomella*. Some areas identifiable as very rich in Agromyzids are Valley of Flowers in the Garhwal Himalaya, Darjeeling-Lebong-Tiesta area in the eastern elevations of Himalaya, and Periyar-Kumili areas of western ghats. *Liriomyza*, *Cerodontha* and *Chromatomyia* are the predominant genera in these regions.

4. Economic impact

The impact of Agromyzidae on economy is felt much more in the developing countries of the tropical belt where species like *M. obtusa*, *C. horticola* and *O. phaseoli* are pests on economically important crop plants while in the temperate zone, though the Agromyzid population is high, they are more of a green house problem (as they are pests on vegetables and ornamental plants in green houses). In India where majority of Indians especially of the lower income groups are dependent on leguminous crops for their protein intake, economic impact due to the infestation of various Agromyzids is considerable. Almost all the leguminous crops have an Agromyzid pest and some of them are of major pest status. The major oil seed of the Gangetic plain, *Brassica campestris* (Sarson) too has a major Agromyzid pest infesting it. Few important pests and their host plants are given in table 2.

In the case of seed borers like *M. obtusa* the loss is direct due to the larva feeding on the seeds. In the case of stem borers like *O. phaseoli* or *M. sojae* the feeding by their immature stages in the internal part of the stem causes external browning of the stem leading to death of plants in extreme cases. The leaf miner infestation is quite

Table 2. Important Agromyzid pests with their host plants and per cent of infestation.

Agromyzids (Status as pest—major)	Host plant	Average plant infestation	Maximum infestation
C. horticola	*B. campestris*	67·41	98·75
	Pisum sativum	58·24	99·05
	Tropaeolum majus	47·75	91·15
	Lathyrus odoratus	40·05	81·67
L. brassicae	*B. campestris*	57·13	83·26
	P. sativum	50·48	77·31
	T. majus	35·71	58·60
	L. odoratus	24·92	34·68
O. phaseoli	*Vigna catiang*	100	100*
	Dolichos lablab	60·00	60·00*
	Glycine max	4–5	4–5
	P. sativum	33·00	39·00
	Phaseolus mungo	37·08	48·08
M. sojae	*G. max*	50·00	—
	Phaseolus radiatus	42·02	50·00
	P. mungo	84·06	90·02
	P. sativum	60·00	—
M. obtusa	*Cajanus indicus*	61·73	100

*Subject to ecological conditions.

high during peak periods on crops like *B. campestris* and their feeding causes wilting and total loss of the leaves in acute cases of infestation.

5. Parasitism

Parasites are the most important factors influencing the Agromyzid populations. Experiments so far have provided conclusive evidence to justify preference of biocontrol methods to chemical control for these pests. Some important parasites reared from the host species are listed below:

Agromyzid hosts	Parasites
M. obtusa	*Euderus lividus* *Diglyphus mandibularis* *Digliphus funicularis* *Tetrastichus atomella* *Euderus agromyzae* *Ormyrus orientalis* *Eurytoma* sp.
M. sojae	*Afrostilba* sp. (Eucoilidae) *Opius phaseoli* (Braconidae)
C. horticola	*Chrysocaris horticola* *Solenotus* sp. (Eulophidae) *Tetrastichus* sp. (Eulophidae) *Opius* sp.
C. syngenisae	*Closterocerus phytomyza*
O. phaseoli	*Opius phaseoli* *Trigonogastra rugosa* *Tetrastichus* sp. *Eurytomidae* sp.
L. brassicae	*Diglyphus indicus*
E. atomella	*Tetracampae indica*

Biological observations on the parasites are limited. The available information indicates that both *O. phaseoli* and *M. sojae* infestations are kept under control by parasites as the percentage of parasitism reaches appreciable levels every season. But this cannot be said about *M. obtusa* or *C. horticola*, both the pest forms reaching alarming levels of infestation each season with no corresponding effective build up of parasites (table 3).

6. Some factors influencing population

6.1 *Rainfall*

A single factor that has profound influence on Agromyzid distribution is the dominant climatic factor—the monsoons. Rainfall in addition to influencing the

Table 3. Yearly pattern of parasitic levels observed in the Gangetic plains and per cent of parasitism.

Month	*Ophiomyia phaseoli* on *Vigna catiang*	*Melanagromyza sojae*		*Melanagromyza obtusa* on *Cajanus indicus*	
		Phaseolus mungo	*Pisum sativum*	Early var.	Late var.
Jan.	—	—	53	—	56
Feb.	—	—	—	—	50·1
March	—	—	—	—	52·5
April	—	—	—	—	—
May	—	—	—	—	—
June	—	—	—	—	—
July	—	8·3	—	—	—
Aug.	—	54·1	—	—	—
Sept.	—	72·2	—	13·2	—
Oct.	46·2	91·7	—	22·0	—
Nov.	94·0	—	—	21·7	—
Dec.	—	—	27·0	—	38·3

Data for individual parasites are not available.

vegetation has short term impact such as destruction of the adult flies, washing away of pupae of species that fall on the ground and killing of the larvae in mines on the leaves. This physical impact has profound influence on the short term population structures of Agromyzids especially in the tropical peninsular India.

Immediate post monsoon collection trips yielded very poor collection and also large number of dead immature stages inside the mines due to mines getting filled up with water. Thus often it was found that one generation itself gets wiped out due to unseasonal rains.

6.2 *Wind*

Wind can also be considered as a physical factor that influences the Agromyzid population. They prefer shady protected area for egg laying. Some of the windy islands of the Andaman and Nicobar island chains are very poor in Agromyzids though their vegetation is very much similar to the western coastal belt with comparatively rich Agromyzid fauna. Presence of hills and peaks on the western ghats provide protective niches and hence though windy western ghats are richer in Agromyzidae.

6.3 *Cultural practices*

In the Gangetic plains where farmers have been reluctant in employing modern cultural methods including rotation of crops, and where monoculture has been in practice for many years the population of species like *C. horticola* and *M. obtusa* infesting mustard crop (*B. campestris*) and Arhar crop (*Cajanus indicus* L.) respectively has gone up to unmanageable levels.

6.4 *Host selection*

Agromyzids are generally host specific. The chain process of host selection can be split and defined into (i) host habitat finding, (ii) host finding, (iii) host recognition, (iv) host acceptance and (v) host suitability. Very little information is available on habitat finding or host finding by Agromyzids. Ovipositor-labella combination is used for host recognition and acceptance. The female fly makes numerous punctures on the plant tissue with the help of the ovipositor. The exuding plant fluid is tasted with the help of labella by the female flies and eggs are laid singly once the host plant is accepted. Only about 8–10% of punctures are deposited with eggs and become eventually ovipositional punctures, the remaining ones being left as pseudopunctures. Misidentification of host plants by female has been observed but is very rare. Males are incapable of making punctures hence often been sharing the plant exudation from the punctures made by the female. Male life span is shorter than the female.

7. Future course of investigations

Investigations into the behaviour of Agromyzids needs much more inputs. The factors requiring special attention are: (i) Organs and devices responsible for host recognition and host selection, (ii) correlation between the structure of these organs and the behaviour of these flies, (iii) recognition of special features in their biology and (iv) study on the interrelationship between special features in their biology and bioecological factors.

Though Agromyzid infestation causes damage to varied plant parts such as root, stem, leaf, seed etc, there is a striking similarity in the organs and devices responsible for host selection. This is mainly due to the fact that in all cases it is the adult fly which is responsible for choosing the host. More investigations are needed into the correlations between structural adaptations for host recognition and selection and the behaviour of Agromyzids.

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Biosystematics of Aleyrodidae (Homoptera: Insecta)

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Abstract. The scarcity of positively associated stages of adults of both sexes and the remarkably little variation shown by the adults of a very few species studied have naturally led to the recognition of the so-called pupal cases, the exuviae of the IV instar nymphs in the matter of generic and specific classification of the aleyrodids—a rather unusual procedure in the taxonomic study of these insects. This stage is the one most often collected and known in the species world over and also possesses a multitude of diagnostic features than in any other stage.

Keywords. Biosystematics; Aleyrodidae.

1. Introduction

The importance of host association in the taxonomy of Aleyrodidae was first stressed by a few workers in the year 1933. Mention may be made of the reported variations in setae in *Bemisia tabaci* (Gennadius) by Husain and Trehan (1933) and in *Aleyrodes lonicerae* Walker by Deshpande (1933). The correlation between the structure of the aleyrodids and that of the leaves upon which they lived has been noticed in a number of aleyrodids from Formosa by Takahashi (1933). Trehan (1940) demonstrated the difference in number and size of the setae of *A. lonicerae* when this species developed on *Rubus* rather than on *Lonicera*, and noted a difference in their development in *Asterobemisia carpini* (Koch) when the latter was reared on blackberi (*Rubus*) rather than on hornbean (*Carpinus*). Russell (1948) stressed that conspicuous difference in the size of setae and development of papillae, and in the size of the anal organ found in some species of *Trialeurodes* was apparently or due largely to changes in the insect environment. More plastic species have been developed on diverse types of leaves and the appearance of such insects may be altered conspicuously by a change of host. Species showing such plasticity with regard to the hairy and glabrous nature of host plants in relation to variations in the morphological features of the pupal cases has been stressed in a number of species occurring in India such as *Acaudaleyrodes citri* (Priesner and Hosney), *Trialeurodes ricini* (Misra), *Bemisia tabaci*, *Dialeurodes bassiae* David and Subramaniam, *Dialeurodes vulgaris* (Singh) and *Pealius simplex* (Singh).

2. Host-correlated variations in aleyrodid taxonomy

Russell (1948) stressed based on her study of specimens of pupal cases of *Trialeurodes vaporariorum* (Westwood) on various host species that considerable variation could be noticed in its structure. This variation was correlated with the structure of the host leaf, on hairy leaves the pupal case bearing large papillae and long setae, and on glabrous leaves, small papillae and short setae. This host-correlated variation has led to the erection of a number of synonymous species. Such host-correlated variations

were observed by her in *T. vaporariorum*, *T. packardi* (Morill), *T. vittata* (Quaintance), *T. abutilonea* (Haldeman), etc.

Russell (1957) synonymised several described species of *Bemisia* with *B. tabaci*. However, Mound (1963) demonstrated experimentally by raising several forms from a single virgin female whitefly by allowing her progeny to develop on different species of plants. He showed that the two forms from tobacco and cassava differed significantly in shape as well as size and it is likely that these variants are induced phenotypically or morphological characters of the host plant leaves such as cuticle irregularity and hairiness. Eastop (1969) further demonstrated that in the case of *B. tabaci* viscous globules appear on the pupal case in the position where the setae will appear and after about 16 h these globules are replaced by large setae. It was thought that the setae may be formed around a thread of wax extruded from the base of the microseta into the globule. He demonstrated that if all the globules are removed from one side of the body, no setae are produced and a normal set develop on the other. It is thought that the setae on genera like *Aleurocanthus* and *Siphoninus* are formed in the normal way before the moult.

Mound (1965) observed the variable shape of the rhachis in specimens of *Acaudaleyrodes citri* (Priesner and Hosney) from populations of Egypt and Sudan. In the same polyphagous species Bink (1983) observed host-correlated variations such as oval to subcircular pupal case, the absence or presence of median tubercles on 1st abdominal segment, sub median setae being from minute to long in nearly every population, etc.

3. Host-correlated variations in Indian Aleyrodidae

David and Ananthakrishnan (1976) and David and Subramaniam (1976) reported on the host-correlated variation in *Trialeurodes ricini* Misra and *B. tabaci*. The observations revealed that the pupal cases developing on hairy leaves are comparatively smaller in size with deeply indented margin, more number of subdorsal papillae and elongated dorsal setae as against large sized pupal cases with smooth margin, less number of subdorsal papillae and very short setae. Alexander (1987) studied in detail the intraspecific diversity in *B. tabaci* on different varieties of cotton. He observed the pupal cases from the varieties of cotton viz Amaravathi, MCU 5, AV 3373/4, ACP 71–42 and L 389 were slightly smaller in size with as many as 8 pairs of dorsal setae whereas those from Sangam, AV 3649, DCH, LKH 71, LPS 141 and LRA 5166 possessed 4–7 pairs of dorsal setae. His study also confirmed that pupal cases from hairy leaves of cotton were much reduced in size than those from non-hairy varieties. Further, the pupal cases from upper surfaces of leaf of cotton resembled the one that was from non-hairy varieties of cotton.

David and Subramaniam (1976) and Alexander (1987) based on a detailed study of *Pealius* spp. showed that the pupal case of *Pealius simplex* (Singh) from the upper surface of leaves of *Ficus glomerata* and *F. bengalensis* was bigger in size with smooth margin and minute subdorsal setae. On the other hand the pupal cases from the under surface of leaves were slightly smaller in size with margin deflexed at several places and long subdorsal setae.

In the case of *Dialeurodes bassiae* the subdorsal setae on the pupal cases were capitate (David and Subramaniam 1976). In a few instances the pupal cases from the

under surface of leaf had elongated, pointed, slender hair like setae in the place of a few of the short capitate setae.

Selvakumaran (1986) observed that the pupal cases of *Dialeurodes vulgaris* Singh from *Jasminum pubescens* differed from *J. sambac* in being smaller in size, irregular and constricted margin, longer cephalic setae; well developed caudal setae; submargin with 10 pairs of minute setae (having 2 pairs of additional setae in mesothoracic and fifth abdominal segment) as against 8 pairs, dorsum and cephalothorax not densely granulated but with distinct median tubercles and vasiform orifice comparatively smaller and notched towards the caudal end.

4. Discussion

It may be seen from the above that a few species belonging to the genera *Trialeurodes* and *Dialeurodes* show remarkable variation in the morphological features on the hairy or glabrous nature of the host plants. Similar intraspecific variations as influenced by host plants has also been reported in the whitefly species viz *A. citri*, *B. tabaci* and *Pealius simplex*. However, the morphological features also show distinct variation when they occur on the upper as well as lower surface of leaves, in the case of *B. tabaci* and *P. simplex* on the same host. These observations emphasise caution in aleyrodid taxonomy and the need for study of a large number of specimens associated with different hosts and establish whether the species is a stable one or capable of showing plasticity.

5. Future areas of research

It will be seen that in aleyrodid taxonomy so far stress has been laid on morphological characteristics of the pupal cases in relation to the hairy and glabrous nature of the host plant and the texture of the leaves. The following areas in relation to biosystematics of this group of insects need immediate attention: (i) Cytotaxonomy of whiteflies, (ii) biochemical approach to taxonomic study of whiteflies, (iii) the biology of the eggs of whiteflies of various taxa and (iv) numerical taxonomic approach.

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Biosystematics of Tingidae on the basis of the biology and micromorphology of their eggs*

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Abstract. The biology and micromorphology of the eggs of 40 species belonging to 26 genera and two subfamilies of Tingidae from southern India have been studied and considered for an assessment of their biosystematics. The oviposition strategy is intimately correlated with the selection of oviposition site on the host plant, determined by the shape and size of the egg and accomplished by appropriately developed ovipositing mechanism involving the structural features of the first gonapophyses. The oviposition pattern is accordingly classified and the eggs are classified on the basis of the nature of development of the chorionic collar cum opercular apparatus. Characterization of the eggs and assessment of their systematic importance have been linked with the origin and evolution of adaptive radiation of oviposition strategies of their egg parasitoids as well. Production of season oriented dimorphic eggs is common among species that oviposit their long operculate eggs vertically in clusters, either into stems or rachis or pistil. Lamina ovipositors preferentially oviposit into the mesophyll horizontally, without cluster formation, on the undersurface of the leaves and the significant reduction in the number of aeropyles of such oval, short operculate eggs could be correlated with the abundance of oxygen supply of the ambient air. More elongate, long operculate eggs in Tingidae, characterized by their multiplicity of aeropyles and vertical oviposition in clusters into stems, rachis and pistil, signify primitiveness, as observed in Cantacaderinae and some large sized Tinginae. Micropyles are absent in tingid eggs, as fertilization occurs before chorion formation and a true spermatheca is wanting.

Keywords. Tingidae; oviposition strategies; opercular diversities; evolutionary trend; biosystematics.

1. Introduction

The pioneering contribution of Southwood (1956) on the classification of Terrestrial Heteroptera on the basis of egg structure and the classic monograph of Cobben (1968) on the evolutionary trends in Heteroptera on the basis of the micromorphology of the eggs, continue to challenge those who try to venture on the biosystematics of any particular family of Heteroptera. Unfortunately, the posthumously published 3 volume treatise of Hinton (1981) on the biology of the insect eggs, but for the third volume comprising of the bibliography, has nothing substantial to offer on the biology of the eggs of Heteroptera. Nevertheless, the contributions of Salkeld (1972) and Haridass (1985a, b, 1986a, b) on the ultrastructure of reduviid eggs have enormously enhanced the scope of future attempts on the biosystematics of Cimicomorpha in general on the basis of the biology and micromorphology of their eggs.

The Tingidae, constituting a minor family of Heteroptera, ever since Johnson (1936) published the first elaborate account of the biology of the *Rhododendron* lace bug, *Leptobversa rhododendri*, has been steadily registering its importance as a

*Contribution No. 44.

weed control agent, as a potential vector of coconut wilt and as a rare example of floral gallmaker. The biology and micromorphology of the eggs of Tingidae has been considered in specific cases of biological studies, attempted by Roonwal (1952) on *Teleonemia scrupulosa*: Stusak (1961, 1968), on *Monanthia symphyti*; *M. rotundata*; *Acalypta marginata*; *A. parvula*; *A. corynata*; *Elasmotropis testacea*: *Catoplatus fabricii*; *Physatocheila dumetorum*; *Deryphysia foliacea*; *D. catoplatus*; *Tingis auriculata*; *T. reticulata* and *T. stachydis*, Livingstone (1959, 1962a, b, 1967a, 1968, 1976) on *Urentius euonymus*; *Monosteira minutula*; *Cadmilos retiarius*; *Tingis buddleiae* and *Dasytingis rudis* and Livingstone and his co-workers (Livingstone *et al* 1981; Livingstone and Yacoob 1983a, b, 1986) on *Pontanus puerilis*, *Agramma* spp. and *A. hupehanum*. The more recent publications of Yaccoob and Livingstone (1983) and Livingstone and Yacoob (1983a, b, 1986) on the egg Parasitoids of a miscellany of Tingidae of southern India provide adequate informations for placing emphasis on the biology of the eggs as a significant aspect of biosystematics. In the present paper, the micromorphology and biology of the eggs of 40 species of Tingidae belonging to 26 genera and two subfamilies of southern India provide the bases for assessing the biosystematics of this family.

2. Oviposition site selection and oviposition strategy

Tingidae are by and large species specific in their host selection (Livingstone 1977). They feed and oviposit on the same host plant. But each species selects its own oviposition site and accordingly their strategies of oviposition vary. All species of southern India whose biologies are known are multivoltine. Even the exotic species *T. scrupulosa*, the *Lantana* lace bug, which is known to be bivoltine in northern India (Khan 1945; Roonwal 1952), is multivoltine in southern India, breeding all round the year, producing more than 12 generations (Livingstone *et al* 1980). Most European species of tingids are known to be bivoltine (Drake and Ruhoff 1965). The British thistle lace bugs, *Tingis ampliata* and *T. cardui* are reported to be univoltine. However, their overwintering phenomenon is not well understood (Eguagie 1972a, b, 1973, 1974a, b).

All the 4 known species of the subfamily Vianaidinae are reported to be myrmecophilous and but for a meagre fragmentary information on the micromorphology of the ovariolar egg of one species (Cobben 1968) their biologies are not known. The gallicolous species of *Copium* and *Paracopium* are anthophagous and they oviposit into the corolla by rotating their eggs in their oviduct at 180°, so that while ovipositing, the operculum could be directed to the interior of the flower bud. The nymphs that hatch enter the space beneath the corolla and make the monothalamus floral cecidium (Darke and Davis 1960; Darke and Ruhoff 1965). This unique rotatory mechanism of the eggs in these bugs is not even vaguely understood from the reports of Fischer (1956), Monad and Carayon (1958), Abraham and Abraham (1971) and Jaeger (1976).

Specificity in oviposition site selection on the host plant is strongly indicated as revealed in table 1 and the manner of oviposition is determined by the oviposition site, as indicated in table 2. The manner of oviposition is further indicated by the specialization of the ovipositor (figure 5). Such specializations become species specific and such an explorative study provides valuable information on the biosystematics,

inter-specific relationships and adaptive radiation of this or any other group of insects.

2.1 *Lamina oviposition*

Depending on the thickness and architecture of the lamina or the calyx, the eggs are inserted either parallel (horizontal) to the leaf surface as in all the grass tingids such as *Aconchus urbanus*, *Agramma* spp. and other monocot tingids such as *Stephanitis* spp. or slantingly into the mesophyll as in the majority of lamina ovipositing species (figure 3J). The degree of obliquity is directly related to the relative thickness of the lamina. Tingids of the verbenaceous plants tend to insert their eggs near to vertical position, similar to those that insert their eggs into stems and pistils. Horizontal deposition in monocot lamina invariably occurs in between parallel veins.

In lamina oviposition, since the eggs are inserted into the substance of the leaf, the upper and lower epidermis turn transparent, and the site of oviposition becomes dechlorophylled, betraying the presence of the egg. All the lamina depositors show varying degrees of preference to specific sites such as dorsal surface, ventral surface, leaf margin and leaf tips (table 1) and majority of them prefer the ventral surface, for safety and protection from direct exposure to sun light. It has also the advantage of the safety of the nymphs from predators and from being directly exposed to insecticides. The first gonapophysis of the lamina ovipositors can be readily recognized by the arrangement of serrations, especially in the grass tingids, for facilitating insertion into the flaccid blades (figure 5 J–N).

2.2 *Rachis/petiole/vein oviposition*

Considerable variations occur in the degree of obliquity of oviposition, depending on the thickness of the substrate. When the eggs are deposited into the midrib they are closer to vertical insertion as in *Pontanus puerilis* (figure 3G) and *Perissonemia ecmeles* (figure 3E).

2.3 *Floral parts oviposition*

There is specificity in the selection of floral parts also. *C. retiarius* selects the calyx (Livingstone 1962b). *D. rudis* (Livingstone 1976), *D. semota* and *Ammianus ravanus* specifically select tender pistil and oviposit in clusters of 3–8 eggs. The eggs hatch before the pistil matures since mature fruits always carry shells of hatched eggs.

2.4 *Stem oviposition*

This is characteristically found in *C. bullita*, inserting its eggs vertically in batches of more than 10 eggs per batch into the tender stems of *Ocimum* spp. Lamina oviposition is also not uncommon in this species, as reported by Samuel (1939) and Sharga (1953). *Phatnoma costalis* alone has been found ovipositing exclusively into the tender stems of *Hibiscus*. In *C. retiarius*, a polyphagous species of north India has *Helianthus* as its primary host in which it oviposits on the sepals and lamina. But

Table 1. Site of egg deposition in Tingidae.

Tingid species	Leaf									Floral		
	Dorsal surface	Ventral surface	Leaf tip	Leaf margin	Midrib	Veins	Inter venous	Petiole	Rachis	Calyx	Pistil	Stem
Aconchus urbanus	–	+	–	–	–	–	+	–	–	–	–	–
Agramma graminii	–	+	–	–	–	–	+	–	–	–	–	–
Agramma hupehanum	–	+	–	–	+	–	+	–	–	–	–	–
Afrotingis phanuelii	+	–	–	–	–	–	+	–	–	–	–	–
Ammianus ravanus	–	–	–	–	–	–	–	–	–	–	+	–
Cochlochila bullita	–	–	+	+	+	–	–	+	+	–	–	+
Corythauma ayyari	+	–	+	+	–	–	–	–	–	–	–	–
Corythauma gibbossa	–	+	–	–	–	–	+	–	–	–	–	–
Cysteochila javensis	+	–	–	–	–	–	+	–	–	–	–	–
Dasytingis rudis	–	–	–	–	–	–	–	–	–	–	+	–
Dasytingis semota	–	–	–	–	–	–	–	–	–	–	+	–
Dictyla karnatica	+	+	–	–	–	–	–	–	+	–	–	+
Dulinius conchatus	–	+	–	–	+	+	+	–	–	–	–	–
Eteoneus cinchonii	+	+	–	–	+	+	–	–	–	–	–	–
Habrochila laeta	+	+	–	–	+	–	–	–	–	–	–	–

| | | |

Table 2. Mode of egg deposition in Tingidae.

Solitary	Batches	Cork formation	Smearing of faecal matter
Aconchus urbanus	*Cochlochila bullita* (30)	*Corythauma ayyari*	*Corythauma gibbossa*
Agramma graminii	*Dasytingis rudis* (3–6)	*Dasytingis rudis*	*Dictyla karnatica*
Agramma hupehanum	*Dasytingis semota* (3–5)	*Dasytingis semota*	*Habrochila laeta*
Corythauma ayyari	*Dulinius conchatus* (2–3)	*Eteoneus cinchonii*	*Naochila minuta*
Cysteochila javensis	*Eteoneus cinchonii* (5–10)	*Phatnoma costalis*	*Phaenotropis cleopatra*
Dasytingis rudis	*Lasiacantha peristrophii* (2–3)	*Ammianus ravanus*	*Stephanitis typica*
Dasytingis semota	*Ammianus ravanus* (2–5)	*Pontanus puerilis*	*Stephanitis cinnamomii*
Dictyla karnatica	*Physatocheila asiatica* (2–3)	*Stephanitis cinnamomii*	
Dulinius conchatus	*Perissonemia ecmeles* (2–4)	*Stephanitis macranthaii*	
Habrochila laeta	*Pontanus puerilis* (2–4)	*Stephanitis typica*	
Haedus grewii		*Teleonemia scrupulosa*	
Haedus yacobii		*Tingis tomentosii*	
Lasiacantha justiciaii		*Urentius euonymus*	
Lasiacantha peristrophii		*Urentius hystricellus*	
Lasiacantha ruellii			
Naochila minuta			
Perissonemia ecmeles			
Phatnoma costalis			
Phaenotropis cleopatra			
Ammianus ravanus			
Physatocheila asiatica			
Pontanus puerilis			
Stephanitis cinnamomii			
Stephanitis charieis			
Stephanitis macranthaii			
Stephanitis typica			
Teleonemia scrupulosa			
Tingis tomentosii			
Urentius euonymus			
Urentius hystricellus			

during rainy season it oviposits also into the tender stems of other selected annuals (Livingstone 1962b). *Dictyla karnatica* also has been observed to oviposit in the tender stems as well as lamina.

2.5 *Comments*

In the case of lamina deposition, the operculum of the egg remains in flush with the surface whereas in the other types where the eggs are inserted vertically, perpendicular to the surface, the operculum and part of the neck of the eggs stick out at varying heights, especially in the case of the long operculate eggs of *P. ecmeles*, *D. rudis*, *D. semota*, *A. ravanus*, *P. puerilis*, *Eteoneus cingonii*, *T. scrupulosa* and *C. bullita*.

Southwood (1956) recognized 4 types of oviposition in Heteroptera—(i) exposed, (ii) semi embedded, (iii) embedded in dead plants and (iv) embedded in live plant tissue. Later, Cobben (1968) summarised these 4 types into (i) lateral superficial, (ii) embedded position and (iii) upright position. He considered the first type as the most general method of oviposition in Heteroptera and the upright vertical placing of eggs has been regarded as the most economical spatial usuage of substrate. This is

often indicated in cluster or clutch formation, as characteristically found in the pistil and stem deposition types in Tingidae and all such eggs are invariably long operculate and elongate. The first gonapophysis of the pistil, stem and rachis depositors are more elongate and spike like with minute serrations facilitating oviposition of such elongate eggs in clusters by piercing the tender substrate (figure 5 O–R).

Vertical oviposition in clusters is associated with parental care (Cobben 1968) and in Tingidae only in *C. bullita*, in which maximum number of eggs per cluster of vertically deposited eggs in the stem has been found, the females are observed periodically inspecting (guarding) the eggs.

Vertical oviposition and cluster formation are considered as indications of primitiveness, an approach towards formation of ootheca and according to Cobben (1968) more advanced type of egg deposition is indicated by eggs being deposited singly, slantingly and haphazardly. It was also suggested that a shift from horizontal to vertical position allows greater dependence on atmospheric air and escape from parasitoids and predators. In Tingidae, though the upright position may render greater dependence on atmospheric air it may not render them any immunity against parasitoid attack, since the egg parasitoids have been observed to oviposit through the operculum and parasitisation of eggs occurs irrespective of the position of oviposition (Livingstone and Yacoob 1986).

Smearing of faecal matter around the exposed opercular surface of the deposited egg, as reported in *Leptoypha mutica* (Dickerson and Weiss 1916) and *Dictyle sufflate* (Livingstone 1962b) occurs in a number of species (table 2). It appears to be a feature characteristic to the lamina ovipositing genera *Dictyla* and *Naochila*. Such smearing serves as a sealing device, protecting the eggs from desiccation and damping but not as a deterrent against parasitoids. It can be readily washed away with water. Though fungus does not attack the material smeared around freshly deposited eggs, there is a dense growth of fungus around the site of oviposition, after the eggs are hatched.

Cork formation around the oviposited eggs (figure 3H) by the plant tissue by reacting with the secretory material of either accessory gland or oviduct, is characteristically met with in long operculate eggs, oviposited either vertically or slantingly into stems, pistil and rachis and in a few lamina ovipositors such as *Corythauma ayyari*, *Stephanitis* spp. and *Urentius* spp. In the case of *P. ecmeles* specialized warty concrescences (figure 4S) have been observed at the concave (dorsal) surface at the collar base. Such materials are found to have been spread on the leaf surface serving as anchoring device as in the case of chorionic serrations reported in *P. puerilis* (figure 5A) (Livingstone *et al* 1981) as well as in *Haedus yacoobii* (figure 5B).

Since oviposition site selection is species specific, such a specificity is indicated by the specialized development of the ovipositor. Thus a lamina ovipositor is recognized by the highly serrated, broad blade like ovipositor and the pistil, rachis and stem ovipositor is recognized by the much elongate, sharply pointed, finely serrated spike like ovipositor. Morphometric analysis by Livingstone and Yacoob (1986) on the eggs of 23 species of Tingidae and their egg parasitoids (*Lathromeromyia* (L.) *tingiphaga*; *Erythmelus empoascae*; *Epolygosita* (E) *duliniae*, *Lathromeromyia* sp. and *Parallellaptera polyphaga*) has provided evidence of variations in the length of the ovipositor of these parasitoids (figure 5 H, I) determined by the relative height of the chorionic collar cum opercular apparatus. It is an example of reproductive polymorphism (ecotypic/biotypic) of a parasitoid species being determined by the height

of the chorionic collar of the eggs of a wide range of host species, throwing challenge to a biosystematist. A mastery over species determination on the basis of morphological characters therefore is recognized as a necessary adjunct to its biosystematics, placing the sovereignty of taxonomy and integrity of the taxonomist in a highly exalted position.

3. Micromorphology of the eggs

Tingid eggs are known from the accounts of Heidmann (1911), Steer (1929) and Leston (1954). Ovariolar eggs as well as oviposited eggs extricated from the plant tissues were mounted in cavity slides either directly in polyvenyl lactophenol or softened in 5% KOH for an hour and cleared in cellosolve (2-ethoxy ethanol) before mounting. Since it is difficult to extricate eggs from grass, the blades carrying eggs (figure 3J) were treated in 5% KOH for a few hours and cleared in cellosolve and mounted. Treated eggs were dissected in lactic acid and mounted in pieces in DPX for micromorphological studies.

The tingid egg (figure 1) is clearly divisible into the body, the chorionic collar and the operculum.

3.1 *Body of the egg*

The body of the egg is invariably smooth, the hexagonal sculpturations not very distinct, with rare exceptions such as *Haedus* spp. In *P. puerilis* (figure 5A) and *H. yacoobii* (figure 5B) the neck region, mostly along the indented side, for a short distance, develops posteriorly directed serrations which according to Livingstone (1976) enable the egg to gain firm grip inside the plant tissue. Such serrations have been reported in the inner surface of the exochorion of *T. buddleiae* also, which has been explained to serve the function of gripping the endochorion which is likely to be pulled out at eclosion and in that event eclosion will not be possible (Livingstone 1967a). In the case of *P. ecmeles*, the occurrence of warty materials at the neck region, mostly on the indented surface, is significant. With a few exceptions, the chorion is light yellowish and in the case of *C. bullita*, *D. karnatica* and *P. cleopatra* even the ovariolar eggs are darkly pigmented. The caudal end of the eggs is rounded in stem and pistil depositors (figure 3P) whereas in most of the lamina depositors it is slightly oblong, permitting easy penetration into the mesophyll (figure 3L).

3.2 *The chorionic collar*

The chorionic rim is the cephalic end of the body of the egg and the chorionic collar is defined as the extension of the cephalic end of the egg beyond the chorionic rim. The junction of the two is the mouth of the egg (figure 1), beyond which the endochorion does not extend. Therefore, the chorionic collar ('limbus chorioni' of Stusak 1961 and 'rim collar' of Cobben 1968) is essentially an extension of the exochorion beyond the mouth of the egg, circumscribing it, as well as the operculum that forms its lid. The chorionic collar is invariably a spongy, reticulate mesh work, traversed by the aeropyles and the height of the collar varies considerably in different species. The

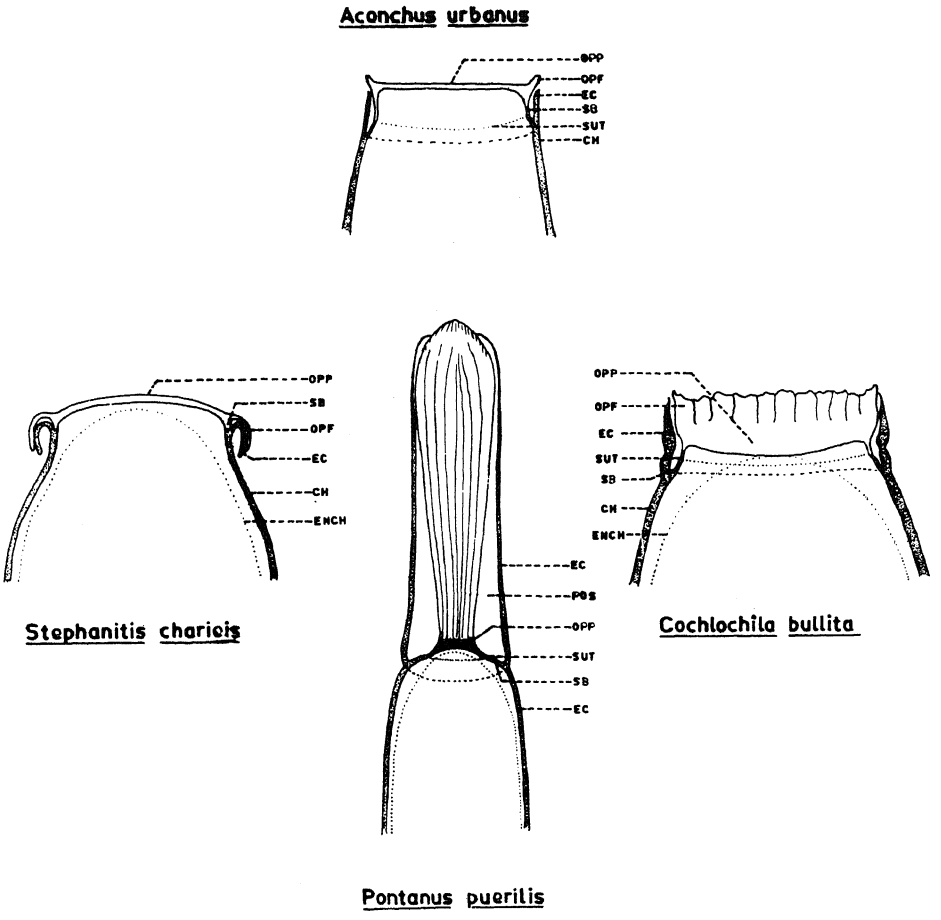

Figure 1. Types of collar-operculum (diagrammatic).

height of the collar is invariably correlated with the height of the opercular crest, but the collar, though intimately associated with the opercular crest, does not fuse as is commonly reported in similar type of eggs of harpactorine reduviids (Cobben 1968; Salkeld 1972; Haridass 1986a). On the basis of the nature of development of the collar cum operculum, tingid eggs could be classified as follows.

3.2a *Short spongy type*: Typically, the chorionic collar here is reduced as a reticulate (spongy) lining of the chorionic rim and the operculum remaining as a lid (figure 3L) without any outgrowth. This type is characteristically found in the eggs of all grass tingids and in all the other lamina ovipositing species the collar, though short, shows different grades of development (table 3). The collar is also plain and spongy as in all the grass tingids as well as *C. ayyari*: *Dulinius conchatus*, *D. karnatica*, *Phaenotropis cleopatra*, *Stephanitis macranthaii* and both species of *Urentius*. It is finely punctate in all species of *Haedus* and *Laciacantha justiciaii* (figure 5B). The aeropyles open to the exterior at the junction of the collar lining and the chorionic rim.

Table 3. Eggs of Tingidae - morphometric analysis (in mm, n = 6, Mean ± SE).

Tingid species	Entire (L)	Entire (W)	Body (L)	Collar (L)	Collar (W)	Sealing bar (Hgt)	Operculum Plate (Dia)	Operculum Flange (Hgt)	Operculum Crest (Hgt)	Aeropyles (L)
Abdastartus atrus	0·592 ± 0·012	0·224 ± 0·014	0·49	0·102 ± 0·012	0·158 ± 0·009	0·008	0·104 ± 0·002	0·01 ± 0·002	0·062 ± 0·005	0·032 ± 0·001
Aconchus urbanus	0·5 ± 0·08	0·184 ± 0·006	0·466 ± 0·011	0·035 ± 0·002	0·112 ± 0·004	0·003 ± 0·001	0·09 ± 0·004	0·017 ± 0·001	—	0·013 ± 0·001
Afrotingis phanuelii	0·322 ± 0·028	0·14	0·273 ± 0·007	0·028	0·112	0·007	0·094 ± 0·007	0·017	—	0·022 ± 0·001
Agramma graminii	0·529 ± 0·011	0·224 ± 0·003	0·466 ± 0·014	0·06 ± 0·005	0·133 ± 0·003	0·003 ± 0·001	0·095 ± 0·002	0·016 ± 0·001	—	0·018 ± 0·001
Agramma hupehanum	0·538 ± 0·015	0·235 ± 0·009	0·436 ± 0·012	0·102	0·12 ± 0·007	0·007 ± 0·001	0·104 ± 0·002	0·104 ± 0·001	—	0·015 ± 0·001
Ammianus ravanus	1·238 ± 0·025	0·205 ± 0·004	0·611 ± 0·006	0·627 ± 0·013	0·142 ± 0·004	0·038 ± 0·009	0·046 ± 0·001	0·072 ± 0·002	0·576 ± 0·022	0·13 ± 0·001
Belenus dentatus	0·716 ± 0·011	0·268 ± 0·005	0·674 ± 0·006	0·042 ± 0·007	0·179 ± 0·009	0·004 ± 0·001	0·146 ± 0·002	0·003	0·015 ± 0·001	0·046 ± 0·002
Cochlochila bullita	0·557 ± 0·002	0·249 ± 0·004	0·485 ± 0·007	0·072 ± 0·008	0·163 ± 0·004	0·005 ± 0·001	0·12 ± 0·001	0·007	0·192 ± 0·002	0·062 ± 0·005
Corythauma ayyari	0·548 ± 0·008	0·231 ± 0·006	0·458 ± 0·006	0·063 ± 0·003	0·13 ± 0·002	0·007 ± 0·001	0·092 ± 0·002	0·007 ± 0·001	0·021 ± 0·001	0·047 ± 0·001
Corythauma gibbossa	0·515 ± 0·006	0·191 ± 0·008	0·452 ± 0·01	0·06 ± 0·008	0·112 ± 0·005	0·007 ± 0·001	0·098 ± 0·003	0·008 ± 0·001	0·018 ± 0·002	0·032 ± 0·001
Cysteochila javensis	0·506 ± 0·011	0·254 ± 0·007	0·459 ± 0·001	0·046 ± 0·003	0·142 ± 0·002	0·004 ± 0·001	0·109 ± 0·003	0·01 ± 0·003	—	0·02 ± 0·001
Dasytingis rudis	0·921 ± 0·023	0·224 ± 0·008	0·574 ± 0·013	0·347 ± 0·029	0·154 ± 0·005	0·041 ± 0·002	0·053 ± 0·02	0·082 ± 0·003	—	0·103 ± 0·013
Dasytingis semota	0·865 ± 0·002	0·274 ± 0·007	0·644 ± 0·008	0·221 ± 0·007	0·182 ± 0·003	0·028 ± 0·002	0·097 ± 0·002	0·168 ± 0·005	—	0·103 ± 0·003
Dictyla karnatica	0·621 ± 0·01	0·243 ± 0·005	0·513 ± 0·009	0·107 ± 0·002	0·144 ± 0·005	0·013 ± 0·001	0·117 ± 0·002	0·003	0·041 ± 0·001	0·066 ± 0·005
Dulinius conchatus	0·476 ± 0·005	0·198 ± 0·002	0·415 ± 0·005	0·061 ± 0·007	0·121 ± 0·003	0·006 ± 0·001	0·084 ± 0·015	0·019 ± 0·002	0·019 ± 0·002	0·039 ± 0·003
Eteoneus cinchonii	0·56 ± 0·006	0·175 ± 0·01	0·415 ± 0·002	0·145 ± 0·004	0·119 ± 0·005	0·032 ± 0·001	0·071 ± 0·002	0·081 ± 0·003	—	0·083 ± 0·002
Habrochila laeta	0·567 ± 0·012	0·221 ± 0·008	0·506 ± 0·013	0·061 ± 0·002	0·147 ± 0·006	0·012 ± 0·001	0·113 ± 0·005	0·003 ± 0·001	0·031 ± 0·001	0·045 ± 0·005
Haedus grewii	0·368 ± 0·005	0·231 ± 0·006	0·332 ± 0·006	0·036 ± 0·006	0·123 ± 0·001	0·007 ± 0·003	0·087 ± 0·001	0·005	0·032 ± 0·004	0·031 ± 0·002
Haedus manii	0·406 +	0·168 +	0·364 +	0·042	0·126	0·003	0·085	0·007	—	0·038
Haedus ruthii	0·369 ± 0·001	0·205 ± 0·009	0·299 ± 0·004	0·07 ± 0·004	0·121 ± 0·009	0·007 ± 0·002	0·075	0·003	0·046	0·027

Table 3. (*Contd.*)

Haedus yacobii	0·434 ± 0·007	0·205 + 0·013	0·38 + 0·01	0·054 + 0·003	0·012 + 0·002	0·007 + 0·001	0·084 + 0·003	0·035 + 0·001	0·073 + 0·002	0·027 + 0·002
Lasiacantha justiciaii	0·62 ± 0·021	0·278 ± 0·001	0·569 ± 0·022	0·047 ± 0·003	0·165 ± 0·009	0·007	0·113 ± 0·006	0·017 ± 0·002		0·035 ± 0·002
Lasiacantha peristrophii	0·565 ± 0·01	0·271 ± 0·006	0·522 ± 0·01	0·042	0·163 ± 0·006	0·005 ± 0·001	0·124 ± 0·002	0·023 ± 0·004		0·027 ± 0·001
Lasiacantha ruellii	0·657 ± 0·013	0·287 ± 0·007	0·623 ± 0·007	0·034 ± 0·003	0·182	0·003	0·121 ± 0·007	0·034 ± 0·002	—	0·034 ± 0·003
Naochila minuta	0·39 ± 0·009	0·296 ± 0·003	0·336 ± 0·006	0·053 ± 0·004	0·114 ± 0·004	0·008 ± 0·001	0·065 ± 0·001	0·019 ± 0·001	—	0·024 ± 0·001
Naochila nigra	0·401 ± 0·003	0·241 ± 0·001	0·362 ± 0·004	0·042 ± 0·003	0·112 ± 0·001	0·004 ± 0·002	0·085 ± 0·001	0·01 ± 0·006		0·02 ± 0·002
Perissonemia ecmeles	1·015 ± 0·013	0·231 ± 0·009	0·583 ± 0·007	0·427 ± 0·014	0·168 ± 0·003	0·016 ± 0·001	0·077 ± 0·003	0·462 ± 0·013	—	0·194 ± 0·006
Phatnoma costalis	0·703 ± 0·001	0·241 ± 0·015	0·61 ± 0·008	0·093 ± 0·008	0·154 ± 0·005	0·013 ± 0·001	0·103 ± 0·008	0·003	0·065 ± 0·001	0·068 ± 0·003
Physatocheila asiatica	0·56 ± 0·005	0·212 ± 0·008	0·532 ± 0·006	0·027 ± 0·006	0·135 ± 0·003	0·006 ± 0·001	0·91 ± 0·002	0·003	0·005 ± 0·001	0·025 ± 0·002
Phaenotropis cleopatra	0·435 ± 0·003	0·224 ± 0·005	0·408 ± 0·006	0·027 ± 0·001	0·121 ± 0·008	0·006 ± 0·001	0·106 ± 0·002	0·003	0·017 ± 0·001	0·025 ± 0·001
Pontanus puerilis	1·055 ± 0·005	0·227 ± 0·004	0·575 ± 0·005	0·48 ± 0·004	0·156 ± 0·002	0·023 ± 0·001	0·081 ± 0·002	0·487 ± 0·004	—	0·236 ± 0·001
Stephanitis charieis	0·375 ± 0·07	0·232 ± 0·09	0·345 ± 0·07	0·03 ± 0·05	0·128 ± 0·04	0·003	0·114 ± 0·004	0·081 ± 0·011		0·076 ± 0·011
Stephanitis cinnamomii	0·381 ± 0·005	0·21 ± 0·006	0·364 ± 0·004	0·017 ± 0·001	0·14 ± 0·004	0·003	0·085 ± 0·001	0·021 ± 0·001		0·027 ± 0·001
Stephanitis macranthaii	0·472 ± 0·003	0·189 ± 0·009	0·434 ± 0·005	0·03 ± 0·001	0·108 ± 0·003	0·041 ± 0·008	0·09 ± 0·001	0·019 ± 0·001	—	0·047 ± 0·001
Stephanitis typica	0·448 ± 0·03	0·217 ± 0·07	0·42 ± 0·02	0·03 ± 0·02	0·086 ± 0·016	0·046 ± 0·007	0·113 ± 0·002	0·017	—	0·032 ± 0·007
Teleonemia scrupulosa	0·649 ± 0·02	0·252 ± 0·006	0·522 ± 0·015	0·126 ± 0·008	0·147 ± 0·003	0·015 ± 0·001	0·085 ± 0·005	0·003	0·087 ± 0·008	0·068 ± 0·001
Tingis tomentosii	0·539 ± 0·01	0·282 ± 0·05	0·508 ± 0·008	0·031 ± 0·032	0·128 ± 0·002	0·007	0·082 ± 0·001	0·003		0·041 ± 0·002
Urentius euonymus	0·448 ± 0·01	0·215 ± 0·007	0·431 ± 0·01	0·035 ± 0·003	0·126 ± 0·003	0·003	0·091 ± 0·003	0·007 ± 0·001	—	0·017 ± 0·001
Urentius hystricellus	0·471 ± 0·005	0·245 ± 0·008	0·429 ± 0·005	0·042 ± 0·003	0·128 ± 0·002	0·004	0·032 ± 0·001	0·008 ± 0·001	—	0·027 ± 0·002

3.2b *Elongate reticulate type*: The collar, when elongated, is invariably differentiated into a basal, more tough and punctate region that gradually differentiates apically into a more elaborate, reticulate and more brittle region which gets easily mutilated at the slightest touch. This apical brittle region corresponds to the problemmatic structureless tissue (Roonwal 1952), net work region (Southwood and Scudder 1956a, b), spongy region (Livingstone 1962a, b, 1967b, 1976) and gas containing meshwork (Hinton 1981). The aeropyles develop apically as definite number of shallow grooves along the reticulate region of the collar and as they proceed basally the grooves become deeper and narrower at the punctate region. As they enter the chorionic rim they become tubes (figure 5F) formed in between the endochorion and exochorion.

3.2c *Elongately frilled type*: The chorionic collar is elongately divided into a definite number of filamentous frills of reticulate texture. It is characteristically developed in *C. bullita*, *T. scrupulosa* (figure 5D) and *P. ecmeles* (figure 3F). In *C. bullita* each frill filament is fusiform, the middle enlarged region is dark, punctate and slightly bulged outward, appearing as circular dark band of the collar (figure 5C). Apically, each frill filament is punctate and an aeropyle in each frill filament is characteristically found. In *T. scrupulosa*, each frill filament is uniformly reticulate throughout its length and the aeropyles characteristically develop, one in between two frill filaments (figure 5D) as grooves along the basal reticulate region of the collar. In *P. ecmeles*, the reticulate apical region of the collar is more elongate and frilled and the frill filaments are often found spread out conspicuously (figure 3F) exposing the long collumn of the operculum sticking out from the centre of the egg. In freshly laid eggs the frill filaments are held together enveloping the collumn (figure 3E). The aeropyles develop from the reticulate basal region of the collar below the level of the frills (figure 4S).

3.2d *The fringe type*: The collar, along with the opercular flange, rolls back on itself circumscribing the chorionic rim (figure 1). This type is characteristically found in all species of *Stephanitis*. In *S. cinnamomii* and *S. macranthaii* the fringe thus formed is compactly invested with the cork that is formed around this region alone (figure 3I). The aeropyles that develop on the collar also follow its course and therefore remain curved.

3.3 *Operculum*

The operculum, forming the lid of the mouth of the egg and being sealed at the base of the chorionic rim by the membrane commonly known as the sealing bar, protect the egg from desiccation and rotting. The operculum can be distinctly described as a plate ('discus operculi' of Stusak 1961) that develops around its rim posteriorly a flange called the sealing bar and on its outer surface it may either be smooth or may develop certain outgrowths. While the sealing bar is consistent in its structure, the outgrowths vary in their structure from species to species. These outgrowths when developed around the rim (periphery) as elongate reticulate tube (figure 3 N–R), is designated as the anterior flange or *collumn* ('limbus operculi' of Stusak 1968) and when the plate on its outer (anterior) surface develops similar

outgrowths, circumscribed by the collumn, such outgrowths remain as central reticulate pillar, designated as *columella* (figure 4T). When the columella and the collumn are clearly defined, the latter as an outer tube circumscribing the former as the reticulate pillar and both together remaining perceptibly elongate, such an egg is described as long operculate egg. When such outgrowths are not well defined they are described as *short operculate eggs.* Long operculate eggs invariably have equally elongate collar and when both the collumn and the collar are juxtaposed, as in the case of most of the *long operculate eggs*, they jointly function as efficient air store. When the collumn and the columella are relatively short and both together remain inseparably fused, it is called *opercular crest.* On the basis of the relative development of the outgrowths of the opercular plate on its exterior, the eggs of Tingidae are classified as given below.

3.3a *Short operculate type*: This type constitutes the majority of the eggs examined and with a very few exceptions such as *C. bullita* and *P. costalis*, all are deposited either in the rachis or petiole or lamina. Here, the operculum is characterized by a short anterior flange (corresponding position of the collumn of the long operculate eggs) traversed by definite number of canals. This flange may be smooth in all grass tingids (figure 4A) and in a few other lamina ovipositors such as *Urentius* spp. In all species of *Stephanitis* (figure 4F), *P. costalia* (figure 4N) and in all species of *Lasiacantha* (figure 4C), the flange is highly reticulose and remains juxtaposed with the corresponding collar. In *S. cinnamomii* (figure 4G) the flange is lobulated.

The outer surface of the opercular plate may also show varying degrees of development of sculpturations in the short operculate eggs.

3.3b *Smooth or plain plate*: The entire surface is smooth as in all species of *Agramma* (figure 4A) or a few rows of hexagonal calls may border it as in *A. urbanus*, *Afrotingis phanuelii*; *Belenus dentatus*; *D. conchatus* and *Lasiacantha* spp. (figure 4D).

3.3c *Sculptured plate*: The entire outer surface of the plate is distinctly punctured by hexagonal sculpturations, as seen in *Urantius* spp. (figure 4E). *Naochila nigra*, *Agramma* spp., *C. ayyari*, *C. gibbosa*, *Lasiacantha ruelii*, *P. cleopatra* and *Stephanitis cinnamomii.* The outermost whorl of sculpturations are more conspicuous as indication of differential sclerotization of the plate.

3.3d *Tuberculate plate*: Each hexagon of the plate develops either hairy outgrowths as in *Tingis tomentosii* (figure 4N), *P. costalis*, *Physatocheila asiatica* and *Cysteochila javensis* (figure 4O) or certain central hexagons develop digitate tubercular outgrowths as in *L. justiciaii* and *Stephanitis charieis* (figure 4H). In the former case these outgrowths camouflage with the tomentose surface of the lamina and make the detection of the eggs at the oviposition site difficult.

3.3e *Crested plate*: The outgrowths from the plate (both flange and columella) as reticulate meshwork, form a compact crest or crown, often traversed by definite number of longitudinal grooves, comparable to the 'Prolatio disci' of Stusak (1961, 1968). In *Haedus grewii* (figure 4M), *Abdastartus atrus*, *D. karnatica* and *T. scrupulosa* (figure 4P) the crest is in the form of meshwork with the grooves running along the periphery. These grooves however, do not open into the interior but since the collar

also is closely juxtaposed with the crest, these grooves remain associated with the corresponding grooves of the collar in the formation of the aeropyles. Stusak (1961, 1968) in *Tingis stachydis* and several other species described the tubes of the crest to be communicating with one another at the base of the crest but not opening into the egg. The number of grooves present on the crest approximates the number of aeropylar grooves of the collar. Thus the grooves on the opercular crest, by their position, are formed on the anterior flange developed from the rim of the opercular plate and the main mass of the reticulate meshwork of the crest is formed from the body of the plate and therefore the flange corresponds to the collumn and the meshwork to the columella of the long operculate eggs. Livingstone (1962b), while describing similar opercular crest in *C. retiarius*, suggested that this structural feature of the crest is an adaptation for humidity regulation. During rainy season the crest appears distended exposing fully the peripheral grooves while during drought it remains collapsed, shrunkan and hard.

All the 4 species of *Haedus* present 4 different types of opercular crest. In the case of *Haedus ruthii* (figure 4L) and *H. manii*, the crest like a crown of hexagonal cells with the chorionic collar encrusting at its base. In *H. grewii* (figure 4K) the crest reveals the peripheral grooves of the flange. An unique type of opercular crest among Tingidae has been found in *H. yacoobi*. Here, the columella is drawn out in the form of a compactly formed hook and the collumn (flange) with the grooves forming the base around it (figure 5B).

The more reticulate type of opercular crest of *T. scrupulosa*, *C. bullita* and *D. karnatica* appears intermediate between short and long operculate types, corresponding structure has been termed as 'prolatio disci' by Stusak (1961, 1968) in *M. symphyti*.

3.3f *Long operculate type*: It is characteristically found in all those eggs with tall chorionic collar. They include *A. ravanus*, *D. rudis*, *D. semota*, *P. acmeles* and *P. puerilis*. In *Eteoneus cinchonii* it is moderately elongate as in *T. scrupulosa*, but structurally both are different. By circumscribing the elongate collumn of the operculum, the chorionic collar encloses a peripercular space (figure 1). In the case of *D. rudis*, *D. semota*, *A. ravanus* and *P. puerilis* the collumn circumscribes the columella as a peripercular cylinder (Livingstone 1976) almost up to the tip and throughout its length it establishes, at regular intervals, transverse septal connections with the latter, as indicated externally by the ribs (figure 4T). By this, a honeycomb pattern of air store is thus established.

The columella is not clearly defined in *P. ecmeles* (figure 4S) and in *E. cinchonii* (figure 4R). In the latter, both the collumn and the columella together expand apically and remain transparent. In all these long operculate eggs, especially in *P. ecmeles*, the collumn is narrow, often constricted basally (figure 4S) thus increasing the area of the peripercular space.

In *P. puerilis*, *D. rudis* and *D. semota* it has been observed that the operculum and the collar together are variable in height, exhibiting seasonal dimorphism (Livingstone *et al* 1981). The eggs oviposited during February are short operculate and those oviposited during October are long operculate. Both types of eggs are inserted vertically, but the former is less exposed, being more deeply buried in the tissue than the latter. Dissections of gravid females of these insects reveal (figure 3C) that the eggs that are ovulated earlier are invariably long operculate whereas those

ovulated subsequently are consequentially short operculate. Consequently, the eggs that are oviposited at the termination of oviposition cycle are always short operculate, thus presenting a typical example of dimorphic eggs. However, the relevance of the production of such dimorphic eggs by these multivoltine species needs further investigation.

The long operculate eggs of Tingidae are comparable to the eggs of Harpactorinae, the chorionic collar of the former and the vail of the latter exhibiting structural and functional similarities. Both have well established periopercular space and comparable opercular elements. In both the cases, eggs are oviposited vertically in clusters. Whereas, in Harpactorinae they are glued to the substratum (leaves, barks, stones and soil) by means of the spumaline secreted by the subrectal glands (Cobben 1968; Hinton 1981), the tingids bury their eggs into the plant tissue and for that reason the ovipositor of Tingidae, especially their first gonapophyses, are more elongate and variously serrated. In Harpactorinae and in all other subfamilies of Reduviidae shortening of gonapophysis is characterised. Though the paired accessory glands are well formed in these tingid species the characteristic cork formation around the inserted egg into the plant tissue due to reaction with the secreted material, necessitates further confirmatory evidences.

3.4 *Respiratory apparatus*

The close juxtaposition of the collar and the opercular flange (collumn), whether high or low, creates an efficient air storing meshwork and the development of the columella from the plate in the crested and long operculate eggs enhances the efficacy of the air storing device, comparable to the plastron of the eggs of terrestrial Heteroptera (Hinton 1981). The creation of the periopercular space in the long operculate eggs, comparable to the 'long double walled respiratory cylinder with fine reticulations' of Cobben (1968), further increases the area of air storage.

Subsequent to the report documented by Carayon (1954) that tingid eggs were fertilized after chorion formation, Southwood (1956) by his cobalt injection technique concluded that the canals that are established by the juxtaposition of exochorion and operculum were micropyles. Later, Stausak (1961, 1968) further emphasised that all the canals of the reticulate collar were micropyles. According to Beament's (1946) definition, all canals that do not have definite opening to the exterior are 'pseudomicropyles', corresponding to the 'Leukart's canals' which are respiratory in function. The 'true micropyles' according to him have definite opening to the exterior and therefore they alone are responsible for sperm transport. The pseudomicropyles are in fact the aeropyles opening into the gas containing meshwork (Hinton 1981) or the aerostatic layer (Cobben 1968) of the exochorion and gaseous exchange with the ambient air is accomplished by these canals. When the periopercular space is present it maintains communication with the ambient air. The fact that the grooves of the opercular flange (collumn) do not communicate with the egg but associated with the corresponding grooves of the collar to form definite canals at the chorionic rim, confirms the formation of aeropyles by the juxtaposition of the collar and flange.

In Tingidae, Cobben (1968) reported to have observed both aeropyles and micropyles. One of the criteria for classification of Terrestrial Heteroptera was the

functional organization of these two canal systems and Southwood (1956) included Tingidae under Cimicomorpha as he considered that both aeropyles and micropyles are present as independent functional entities in their eggs. Hinton (1981) contended that all species of tingids have two micropyles, one on either side (right and left) in all, except in *Cantacader infuscata* in which the two are arranged close to each other on the ventral side.

A micropyle is recognized from the aeropyle by its sharp curve beneath the inner surface of the shell, to open more anteriorly in the shell. In the aberrent myrmecophilous tingid, *Anommatocoris*, a few aeropyles also have been reported to curve into the air containing meshwork in a similar fashion as the micropyles (Cobben 1968). But none of these authors have recognized a distinct opening of the so called micropyle to the exterior at the chorionic rim. Southwood and Scudder (1956a, b) and Stusak (1961, 1968) considered all the visible canals as micropyles and for that reason they failed to recognize any aeropyle in the eggs of a number of species of tingids they examined.

A close examination of the chorionic architecture, collar and operculum of all the 40 species of Tingidae examined does not provide any conclusive evidence of the existence of a canal identical to the micropyle. It is also conclusively established that the curvature of the tube as a diagnostic feature of micropyle is not only arbitrary but also not tenable in Tingidae, since curvature of aeropyles is met within *Anommatocoris* as well as in almost all the long operculate eggs (figure 5F).

True micropyles are reported to be absent in Anthocoridae and Cimicidae and in these two families fertilization took place before chorion formation (Cobben 1968). Livingstone (1967a, b) provided histological evidences in favour of fertilization taking place in Tingidae before chorion formation. In *T. buddleiae* he has confirmed that the paired accessory gland opening into the bursa does not receive sperms, as against the pseudospermatica notion maintained by several workers (Carayon 1950; Southwood 1956; Pendargrast 1957; Draka and Davis 1960) and that the spermatozoa in this polygamous insect are received first in the expanded region of the two lateral oviducts that act as 'primary receptaculum semenis' and from thence they are transported to all the 7 pedicels on each side that act as 7 'accessory sperm reservoirs'. Similar histological evidence has been provided by sections of the ovarioles of *Habrochila laeta* also (figure 3D). Whenever sperms are found either in the pedicels or in the oviducts, the follicular epithelium of the basal oocytes, having clear ooplasm, are found to have not even initiated vitellogenesis. This may also lead to the suggestion that the entire physiology of vitellogenesis and chorion formation, occur as a succession of events following fertilization. Earlier, Kullenburg (1946, 1947) reported that in mirids sperms are stored in the pedicels much earlier to the formation of chorion. Miridae are known to be closely related to Tingidae in several respects especially in the biology of their eggs (Darke and Davis 1960; Cobben 1968).

Regarding the nature of the aeropyles, considerable variations exist in Tingidae. Branching of the aeropyles inside the egg has been reported in *L. rhododendri* (Johnson 1936) and *T. scrupulosa* (Roonwal 1952). Such branching has been considered as 'extension canals' by Livingstone (1976) in *D. rudis* and by Livingstone *et al* (1981) in *P. puerilis*. They are in fact extension grooves (figure 5F) and not canals. In *A. ravanus* also they are present but not in *P. ecmeles*. Therefore, such extension canals commonly occur among long operculate eggs. However, the accessory reservoir on the chorionic rim as part of the aeropyle, as reported in

T. buddleiae by Livingstone (1967a), has been traced only in *T. tomentosii*, suggesting that this is a characteristic feature of the genus *Tingis*.

Variations in the length of the aeropyles in an egg itself in the long operculate eggs have been reported in *D. rudis* (Livingstone 1976) and *P. puerilis* (Livingstone *et al* 1981), the more elongate principal aeropyles (APP) reaching almost the full length of the collar and the short accessory aeropyles (APA) stopping at the basal punctate region of the collar. But in the aerostatic layer of the chorion both short and long aeropyles show the usual curvature.

The number of aeropyles vary considerably in tingid eggs (figure 2) and is significantly numerous in the long operculate eggs. In the short operculate eggs they are less than 20 and in all the grass tingids they are significantly not more than 6, not distributed at equidistance around the chorionic rim, as they are in most other species. It is also significant that in most of the long operculate eggs the aeropyles are arranged in groups of 3–6. In *T. scrupulosa* and *C. bullita* their number correspond to the number of collar frill filaments.

In Tingidae the aeropyles in all the species, whether with or without extension grooves, terminate in the aerostatic layer inside the egg shell.

3.5 *Sealing bar*

The term sealing bar, representing the posterior flange of the opercular plate, that

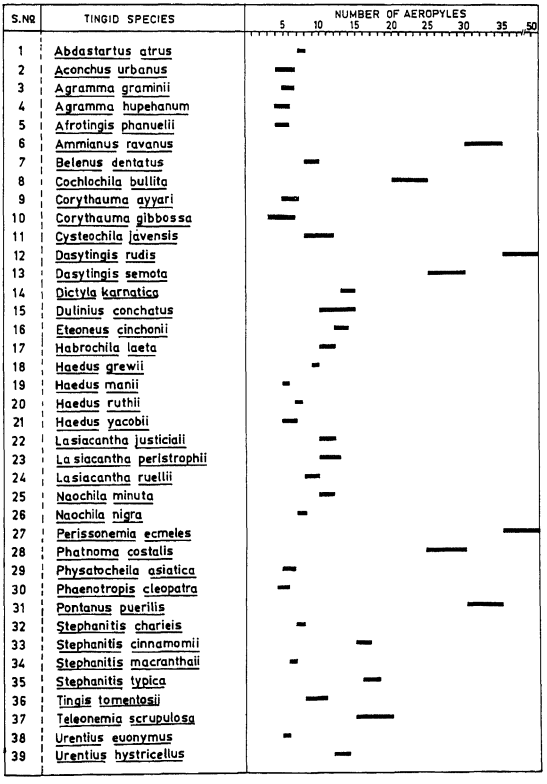

Figure 2. Eggs of Tingidae—numerical distribution of aeropyles.

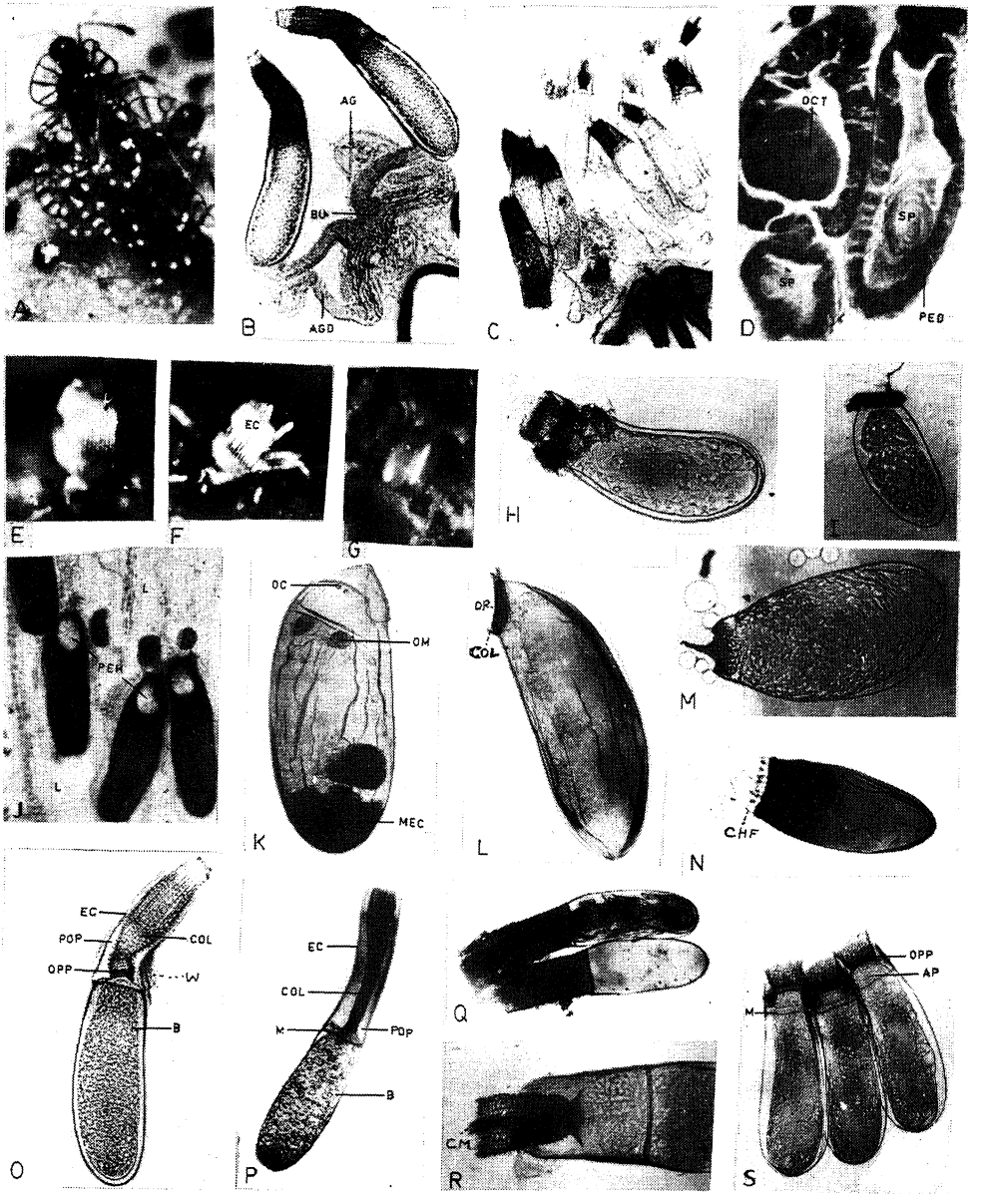

Figure 3.

seals the plate to the base of the chorionic rim, was first coined by Beament (1946) and subsequently by Southwood (1956) and Southwood and Scudder (1956a, b). In Tingidae, Stusak (1961, 1968) described the sealing bar to be composed of two components, the opercular posterior flange proper named as 'basis operculi' and the chorionic component named as the 'claustrum'. At the time of eclosion, the operculum along with the basis operculi has been reported to snap off from the claustrum. Roonwal (1952) and Livingstone (1967a, b, 1976) indicated a line of weakness or suture, corresponding to the junction of the 'basis operculi' and

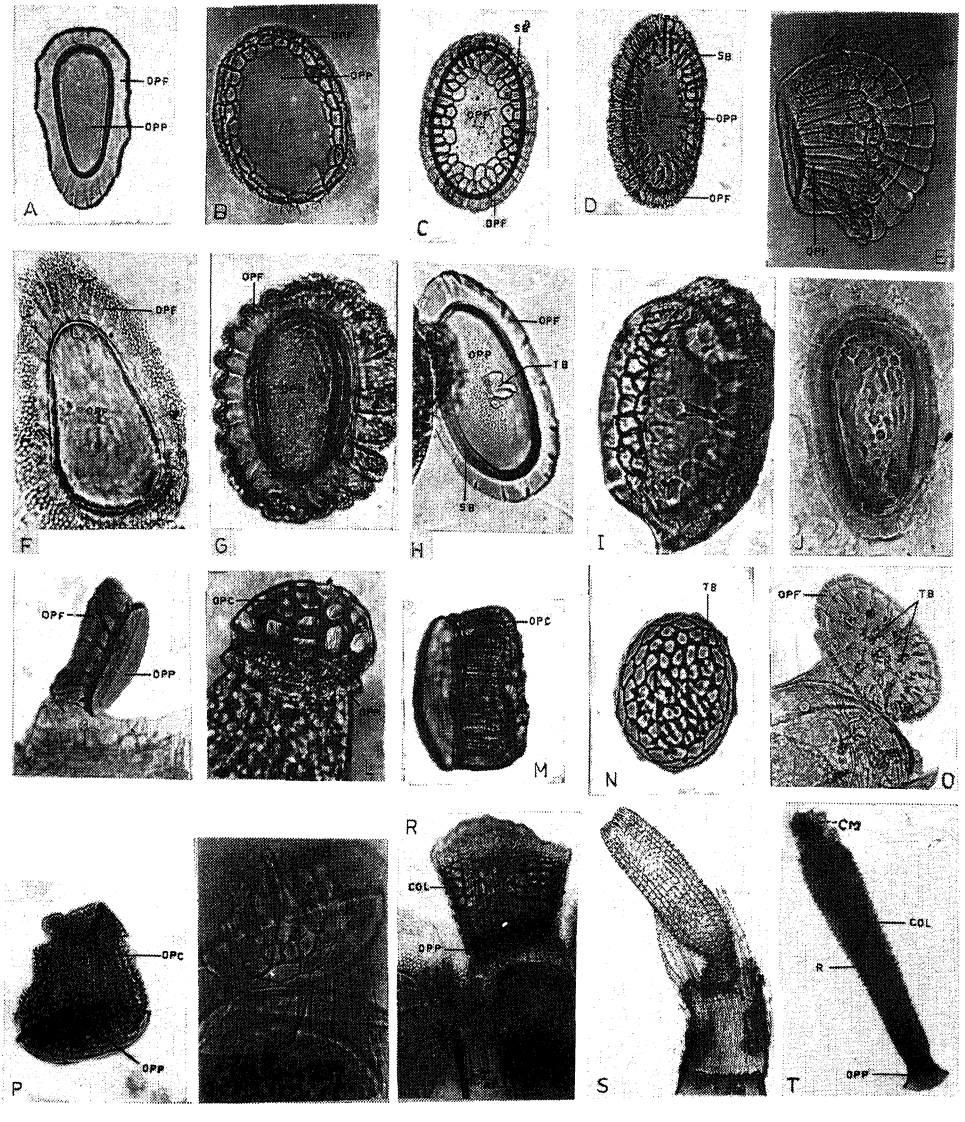

Figure 4. Operculum of a few selected species of Tingidae. **A.** *A. hupehanum.* **B.** *U. euonymus.* **C.** *Lasiacantha peristrophii.* **D.** *L. justiciaii.* **E.** *Urentius hystricellus.* **F.** *Stephanitis typica.* **G.** *S. cinnamomii.* **H.** *S. cherieis.* **I.** *L. ruellii.* **J.** *S. macranthaii.* **K.** *C. ayyari.* **L.** *H. ruthii.* **M.** *H. grewii.* **N.** *T. tomentosii.* **O.** *C. javensis.* **P.** *T. scrupulosa.* **Q.** *P. asiatica.* **R.** *E. cinchonii.* **S.** *P. ecmeles* with the warty concrescences at the neck. **T.** *A. ravanus* with the columella protruding.

'claustrum' of Stusak. In all the 40 species of Tingidae examined, the sealing bar (figure 1) has been found to be the fusion product of the posterior flange of the opercular plate with the anteriorly directed short, more thick flange of the chorion on its inner surface, developing at the base of the chorionic rim. By this fusion, a notch is formed at the base of the chorionic flange in between the rim and the flange which was earlier considered as the place of fusion of the sealing bar

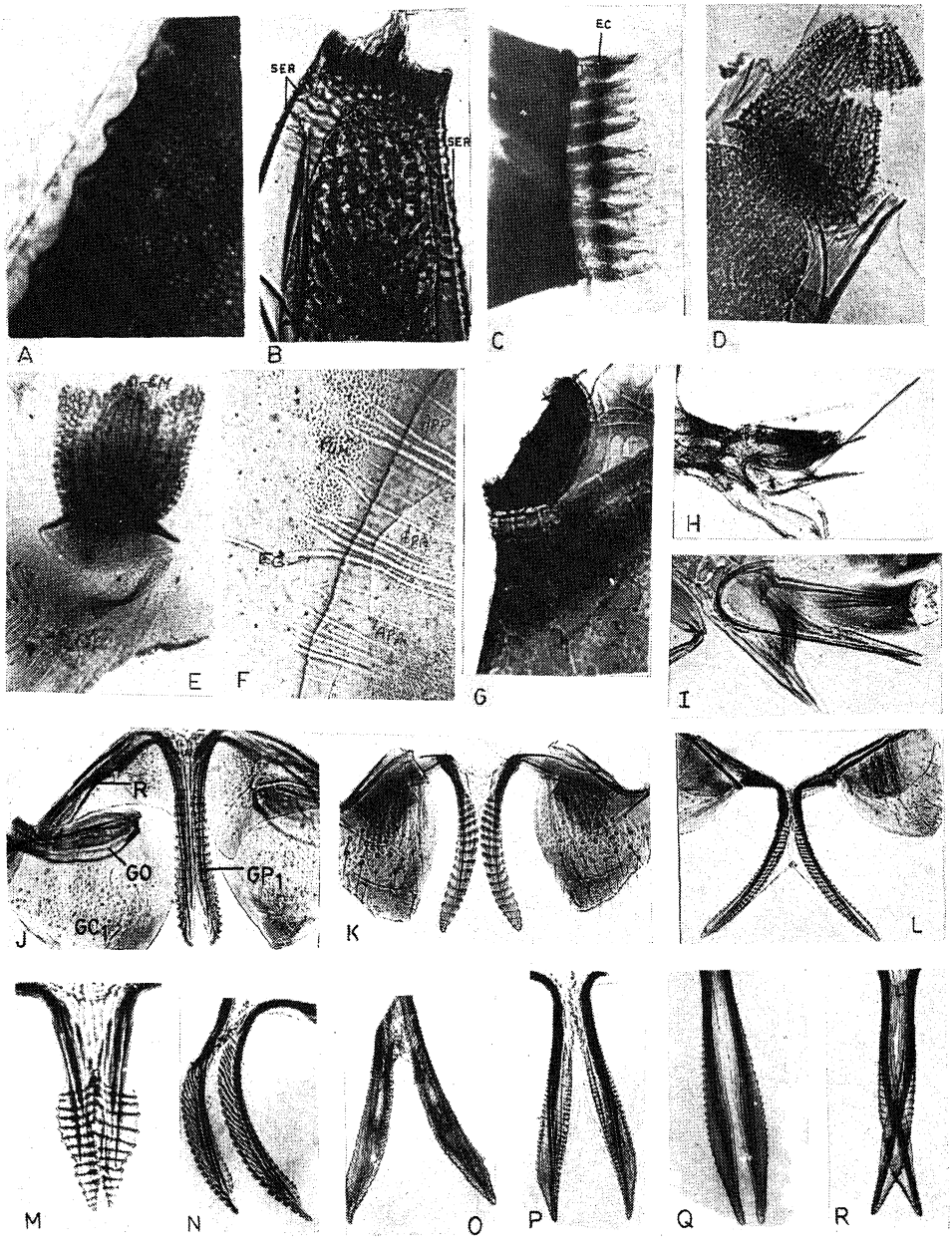

Figure 5.

(Livingstone 1967a, b, 1976). At the time of eclosion, the opercular component of the sealing bar detached itself from the chorionic component and therefore the term suture (SUT) of the sealing bar was found more appropriate to designate the junction of the two components.

4. Biosystematic considerations

Leston *et al* (1954) and Drake and Davis (1960) confirmed Pruthiś (1925) conclusion that the male genitalia of Tingidae is bipartite, resembling that of the Reduviidae and placed both these families closer under Cimicomorpha. Carayon (1954) and Pendergrast (1957) on the basis of the occurrence of 'pseudospermatheca' concluded that Tingidae, Nabidae and Reduviidae are closely related. Southwood (1956) on the basis of the arrangement of the aeropyles and micropyles included Tingidae among Cimicomorpha. Following these earlier revelations Cobben (1968) and Hinton (1981) had taken for granted that Tingidae as a cimicomorph. However, Livingstone (1967) in *T. buddleiae* categorically maintained that the male genitalia is tripartite (a condition declared by Pruthi (1925) as primitive) and a pseudospermatheca is non-existant. The total absence of micropyles, consequential to fertilization taking place before chorion formation, alienates Tingidae from most other families of Cimicomorpha, including Reduviidae that retain both micropyles and aeropyles.

But the absence of micropyles alone does not justify the creation of Tingidae as a major systematic group along with Anthocoridae, Cimicidae and Miridae because several other characters, especially of the cephalic end of the eggs, Tingidae and

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Figure 5. A and B. Neck region of the egg showing serrations of exochorion. **A.** *P. puerilis.* **B.** *H. yacoobii.* **C.** *C. bullita.* Cephalic end of the egg shell showing the central darkly pigmented bulging of the collar frill filaments. **D.** *T. scrupulosa.* Cephalic end of the egg showing collar frill filaments, opercular crest and aeropyles. **E.** *D. rudis.* Short operculum showing collumn and the columella. **F.** *P. puerilis.* Cephalic end of the egg shell showing bundles of principal (long) and accessory (short and slender) aeropyles and their extension grooves entering the aerostatic layer of the egg. **G.** *P. cleopatra* cephalic end of the egg showing aeropyles. **H.** *P. polyphaga.* A mymarid egg parasitoid showing reproductive polymorphism. Here a biotype parasitoid of *D. rudis* showing very slender and more elongate ovipositor. **I.** *P. polyphaga.* The ovipositor of another biotype parasitoid that parasitises the short operculate egg of *Cysteochila javensis.* **J–R.** Nature of first gonapophysis of various species according to the oviposition site. **J and K.** Grass tingids *A. urbanus* and *A. hupehanum* showing highly serrated, blade like first gonapophysis (GC_1, Gonocoxa; GO, gonoplac; GP_1, gonapophysis. R, ramus). **L–N.** *S. charieis, A. phanuelii* and *P. cleopatra*—all are lamina ovipositors. **O–R.** Stem, pistil and rachis ovipositors showing finely serrated more elongate spike like first gonapophysis. **O.** *P. costalis.* **P.** *C. bullita.* **Q.** *P. puerilis.* **R.** *A. ravanus.*

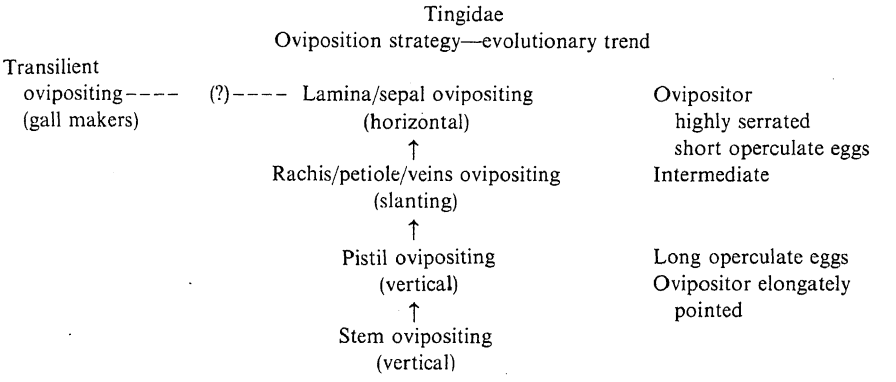
(*Abbreviations*: AP, Aeropyles; APA, accessory aeropyle; APP, principal aeropyle; B, body of the egg; BU, Bursa copulatrix; C, cork; COL, collumn; CM, columella; CH, chorion; CHF, chorionic collar frill filament; EC and ECH, exochorion; ENCH, endochorion; EXG, extension groove; L, leaf, M, mouth of egg; OC, ocelli; OcT, oocyte; OM, compound eye of the parasitoid; OPC, opercular crest; OPF, opercular flange; OPP, opercular plate; PED, pedicel; PEH, parasitoid emergence hole; POP and POS, peripercular space; PUN, punctations of the aerostatic layer of the chorion; R, rib; SB, sealing bar; SER, serrations; SP, sperm bundle; SUT, suture; TB, tubercular process; W, warty concrescence).

Reduviidae share in common. The basis of classification of the eggs into long and short operculate types; the structural similarities of the chorionic collar and its association with the collumn (flange) of the opercular plate and in the creation of a periopercular space; the pattern of distribution and arrangement of the aeropyles; vertical oviposition of eggs in clusters and multiplicity of the aeropyles in long operculate eggs etc could be considered as major such characters that both Tingidae and Reduviidae share in common.

One of the remarkable features, apart from the shape of the eggs, that could be correlated with the systematics and evolution of eggs in Tingidae is the reduction in the number of aeropyles corresponding with the reduction in the height of the chorionic collar which is in turn correlated with the relative obliquity of oviposition, depending on the texture of the host tissue. Long operculate eggs that are vertically deposited in clusters invariably have more spherical caudal end and their cephalic end, almost upto the base of the chorionic rim, protruding far above the site of oviposition. These eggs are characterized not only by the higher numerical density of their aeropyles opening into the periopercular space but also by their dimorphic condition (principal and accessory aeropyles), in marked contrast with the eggs of the short operculate lamina oviposition type. Drastic reduction in the number showing a tendency towards asymmetrical arrangement of the aeropyles on the chorionic rim, with the emanipation of the collar from the operculum, could be correlated with the direct exposure of the aeropyles to the ambient air, close to the surface of the leaves that are actively engaged in photosynthetic process. In the majority of such cases, the under surface of the leaves is preferred.

Interestingly, *P. costalis*, a representative of Cantacaderinae, inspite of the fact that its opercular height is quite insignificant, when compared with the long operculate eggs, the number of aeropyles is significantly higher when compared with all other eggs having similar type of operculum. It is also seen that the aeropyles in *P. costalis* are arranged uniformly spaced around the chorionic rim while in most other vertical depositing species they are grouped in bundles. In the other intermediate types such as *C. bullite* and *T. scrupulosa* their number corresponds with the number of frill filaments.

Drake and Ruhoff (1965) considered Cantacaderinae as a homogeneous group, having more number of fossil species and with a combination of characters that distinctly set them apart from other subfamilies. It is suggested that *P. costalis* represents a more generalized condition of egg types in Tingidae, characterized by vertical oviposition in the stem, with cork formation around the collar and with numerous long aeropyles, showing no trace of alignment into groups. The long operculate type of eggs of non-lamina ovipositors, with the formation of periopercular space and grouping of aeropyles and their differentiation into principal and accessory types could be therefore considered as a specialization in vertical oviposition. The lamina ovipositors, having a tendency to oviposit horizontally into the mesophyll, with their sharply serrated ovipositor, could be considered as more evolved, as it is further evidenced by the drastic reduction in the number of aeropyles. The grass tingids of the genera *Aconchus* and *Agramma* are therefore more highly evolved among the lamina ovipositors. Thus the Cantacaderinae constitutes the primitive subfamily of Tingidae from which the various genera of the subfamily Tinginae have derived specializations in their egg micromorphology and oviposition strategy. Intermediate conditions are found in various genera of Tingidae (scheme 1).



Scheme 1. Progressive reduction in the number of aeropyles.

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Figure 3. **A.** *H. laeta* the *Barleria* tingis. Copulating pair. **B.** *P. ecmeles.* Ovariolar eggs with accessory glands arising from the bursa. **C.** *D. rudis.* Ovarioles showing dimorphic eggs: proximal long operculate eggs and distal short operculate eggs. **D.** *H. laeta* L.S of ovarioles showing basal oocyte with clear cytoplasm and sperm bundle in the pedicel. **E.** *P. ecmeles.* Oviposited egg (vertical) showing chorionic frill filaments folded. **F.** *P. ecmeles.* Oviposited egg showing the frill filaments spread out exposing the collumn of the operculum. **G.** *P puerilis.* Two oviposited eggs in the rachis of teak leaf. **H.** *P. costalis.* Oviposited egg with cork formed around the chorionic collar and rim. **I.** *S. cinnamomii.* Oviposited egg showing fringe type of chorionic collar. **J.** *P. cleopatra.* Eggs oviposited horizontally in the mesophyll. The opening in each egg is the emergence pore of the egg parasitoid. **K.** *A. hupehanum* (a grass tingid). Short operculate egg (parasitised). **L.** *A. urbanus* egg, a grass tingid. **M.** *H. yacoobi.* An oviposited egg showing the opercular crest (Columella) being drawn out in the form of a hook. **N.** *C. bullita.* A hatched egg showing the frill filaments of the chorionic collar. **O.** *P. ecmeles.* An entire egg (long operculate). **P.** *A. ravanus.* An example of long operculate egg. **Q.** *P. puerilis.* Two deposited eggs. **R.** *D. rudis.* A short operculate egg. **S.** *E. cinchonii.* A batch of 3 ovulated eggs.

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Biosystematics in lepidoptera and its importance in forest entomological research*

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Abstract. Lepidoptera constitutes one of the dominant groups of insects in the forest ecosystem, both in terms of species diversity as well as their economic importance. Segregation of taxa in this order is mainly based on the external morphological characters at supraspecific and specific levels, although the morphological details of genitalic armature are also currently being used. More recent trends in the systematics of this group include studies of their ultrastructure, biochemistry, karyology, biometry, cytogenetics etc.

Lepidopterans differ in their habits and habitats, each species having its characteristic habitat requirements, but very sensitive even to slight changes in the environment. As a result, members of different populations which are subject to extrinsic factors affecting the habitats, host-plants or associated organisms exhibit considerable variation. Besides, changes are also brought about by intrinsic factors like parthenogenesis, intraspecific hybridization, changes in the genetic constitution of individuals, etc, which result in a high degree of intraspecific variability within a population.

Keywords. Biosystematics; lepidoptera; Forest entomology.

1. Introduction

Identification of species is basic to any biological research and with this objective, many systems of classification have been proposed from time to time. Of these, the dominant system of classification is the Linnaean system based on the structural details of organisms. According to an estimate by Blackwelder (1964), about 4,000,000 kinds of organisms are classified under this system and it has provisions to include more taxa that are described from time to time. This has been qualified as an 'omnispective' classification. The Linnaean system has undergone considerable modifications in its principles as well as methodology with the advances in the taxonomic research.

2. Development of the biosystematic approach in taxonomy

In the Linnaean system, organisms were classified on the basis of their structural similarity and the structural details of a single specimen representing a population formed the basis for 'species' descriptions. However a species consists of individuals which vary in their morphological details due to individual differences in their genetic make up. In addition to this, there are various extrinsic as well as intrinsic factors that cause variations among the members of a population. The evolutionary concepts of Darwin gave a scientific basis for such variations and 'species' came to be considered as a dynamic assemblage of individuals of varying phenotypes and consequently, species descriptions were formed on the basis of characteristics drawn

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from a group of individuals. Taxonomy thus lost much of its subjectivity and became more objective and theoretical.

It is generally recognized that the more two organisms are similar, the more will they be related in descent. In other words, closely related organisms were expected to possess more characters in common. This led to the proposal of classifications based on a study of a large number of characters which were unselected and unweighted. The results of this purely phenetic approach were not satisfactory, since in such schemes, there were always the danger of the inappropriate characters swamping over the appropriate ones, resulting in unnatural classifications (Blackwelder 1964).

Occurrence of sibling species, seasonal forms, host races as well as allopatric and sympatric variants create confusion in establishing the taxonomic relationships in many 'species groups' using the morphotaxonomic approach. The correct ranking of such variants is often very much needed in applied entomological research, since such variants behave entirely different from species to which they are often associated. As a result, a study of living organisms in relation to its environment has become necessary. Taxonomy thus became more biologically oriented and this led to a remarkable interplay of ideas involving biometry, ecology, biochemistry, genetics etc into the taxonomic schemes. The term 'biosystematics' is applied to such classificatory schemes.

3. Classification of Lepidoptera

Lepidoptera which include butterflies and moths, is one of the most advanced groups of insects and are of great economic importance as pests, pollinators and as objects of aesthetic value.

Taxonomy, in most lepidopteran groups is still in a very primitive state. In most cases, segregation of taxa is based on the morphological details of the head appendages, venation of the wings, structure of legs etc, at the higher taxonomic levels, while the general colour and wing pattern are used for species separation, although, considerable progress has been made recently in arriving at a more stable classificatory scheme using newer characters like the morphological details of the genitalia.

3.1 *Role of biosystematic studies in lepidopteran taxonomy*

Any attempt to discuss the role of biosystematics in the classification of Lepidoptera should consider their biological as well as behavioural characteristics, since the manifestation of even slight changes in these characters among the members of a species are often one of the first indications on the occurrence of intraspecific variants.

Majority of Lepidoptera being phytophagous, depend on plants for sustenance. Some of them can feed on a variety of host plants while some show considerable specificity in host selection. For instance, the toon shoot borer, *Hypsipyla robusta* (Phycitidae), the teak defoliator, *Hyblaea puera* (Hyblaeidae), the teak carpenter worm, *Cossus cadambae* (Cossidae), the paddy caseworm, *Nymphula depunctalis* (Pyraustidae) etc show specificity to certain plants, while the bagworm *Eumeta crameri* (Psychidae), the teak sapling borer, *Sahyadrassus malabaricus* (Hepialidae), the castor fruit borer, *Dichocrocis punctiferalis* (Pyraustidae), the flour moth, *Cadra*

cautella (Phycitidae) and the grain moth, *Crocera cephalonica* (Galleriidae) are polyphagous and can develop on a variety of hosts.

Lepidopterans show considerable diversity in its habits. Even the same family may contain species having diverse habits. There could be leaf feeders, stem borers, fruit borers and so on. Even among those with a more or less similar habit, there could occur many variations in their relative behaviour. For example the foliage feeders may include species that feed exposed or concealed. Similarly the stem borers may include species that prefer to bore into the top shoot portion, or the basal portion of the stem, or in the roots.

The adults normally feed on the nectar of phanerogamic plants. However some species prefer to feed on the juice of over-ripe fruits (*Euthalia garuda*, *Othereis* spp.); on the liquid portion of animal excreta (some butterflies); on the lachrymal secretion of vertebrates (*Lobocraspis griseifusa*); or even on blood (*Calyptra eustrigata*). Such diversification in the habits of Lepidoptera is in response to the nutritional requirements of the particular species and to ensure better survival chances for it.

Being highly mobile organisms, the chances of Lepidoptera in getting distributed over a wide geographical region are relatively high. Large scale migration of many butterflies and moths have been reported from many parts of the world (Ford 1972). In addition to this, there are also chances of their accidently getting distributed through commerce, especially with the recent advancements in the transport system.

Changes in the climatic or edaphic conditions are known to affect the biological processes of living organisms. For example, the duration of life cycle in the carpenterworm, *Zeuzera coffeae* is reported to vary in different climatic zones (Beeson 1941). Similarly, many lepidopterans (Psychidae) can remain dormant as larvae or pupae over long periods especially during adverse weather conditions. Instances of reproductive diapause have been reported in the nymphalid butterflies, *Speyeria coronis* and *S. zerene* in California. In the females of these butterflies, a delay in the ovarian development occurs during the warm summer season, which delays the onset of oviposition until late summer or early fall thus decreasing the exposure time of the overwintering first instar larvae to dessicating conditions (Sims 1984). Studies on the cabbage butterfly, *Pieris rapae* have shown that the food plant on which this insect was previously feeding influenced its further selection of the same host plant (Hovanitz and Chang 1962). Brakefield and Larsen (1984) have reported the occurrence of seasonal forms, in butterflies that occur in habitats characterised by changing environmental conditions. Similarly, in a study of the bagworm, *Pteroma plagiophleps* (Psychidae), Mathew (1986) reported the occurrence of 6 types of variants. Difference in the general colouration coupled with a certain extent of host preference has been noticed in populations of *Atteva fabriciella* (Yponomeutidae), collected on *Quassia indica* and *Ailanthes triphysa* respectively (Mohana Das K, unpublished results). Since living organisms are the physical manifestation of a series of continuing changes in the environment, such changed conditions are likely to affect the biological characteristics of organisms, which may be reflected in their phenotype.

Besides changes in the phenotype, and the relative host preference, Lepidoptera may also show variations in its behaviour on different host plants. For example, the cotton (*Gossypium* spp.) leaf webber, *Sylepta derogata* (Pyraustidae) usually feed singly within folded leaves. It makes only a single fold per leaf on the latter host. However, on balsa (*Ochroma pyramidale*), which has broader leaves, several folds

may be seen on the same leaf, each harbouring a larva (Mathew 1980). The reasons for such host associated behavioural responses remain to be worked out but probably be dependent on the texture of the foliage, its chemical nature, structure of the ingestive organs of the insect as well as its digestive physiology.

Lepidoptera also exhibit changes in response to different geographical regions. Occurrence of distinct geographical races have been recorded in many butterflies and moths. Such geographical races differ only slightly and their segregation is often difficult using the conventional taxonomic methods. Coexistance of closely similar species or intergradation of species in the areas of contact has also been reported. Hudson (1973) stated that there are about 200 species of moths belonging to the genus *Euxoa*, many of which bear close similarity rendering recognition difficult. In a study on the paddy leaf roller, *Chaphalocrocis medinalis*, Mathew and Menon (unpublished results) came across a new species which could be identified only by a detailed study of the genitalic morphology.

Besides the host associated and geographical variations mentioned above, Lepidoptera may also offer variations due to gene mutation. Since in the classical taxonomy, identification is based on the characters of the preserved specimens, such variants might get misidentified as distinct species. Only by studies with the living organism, it is possible to ascertain their taxonomic relationships and the role of biosystematic studies in Lepidoptera is mainly to distinguish such intraspecific and interspecific variants.

3.2 *Progress so far made on biosystematics in Lepidoptera*

As stated previously, recognition of intraspecific categories become important while comparing different populations of a species that show clear-cut host specificity or for distinguishing variants that occur in a geographical region or within the population of a species. To facilitate this, a number of studies have been made in several areas, such as micromorphology, cytology, genetics, biometry and biochemistry. A short summary of the progress in some of the above fields is given below.

3.2a *Micromorphology*: Application of scanning electron micrography has made it possible to locate and evaluate a wide variety of micromorphological characters previously unstudied, with a view to generate as many characters as possible for computer analysis. These characters were selected from the different life history stages and mainly include studies on the chorionic sculpture, structure and position of aeropyles, primary cells etc of the eggs (Byers *et al* 1975; Salkeld 1973, 1976); the structure of sclerotized and pigmented areas especially of the prothoracic shield, anal plate as well as the pinacula (Mutuura 1980) and the morphology of the pyloric portion of the hindgut in the larvae; the microstructure of head and genitalic appendages (Davis 1986), the structure of spermathecal cells, as well as the number and structure of chromosomes in the adult. Munroe (1972) reported the occurrence of a tuft of specialised scales on the head 'chaetosema' in pyralids and used this character for segregation of suprageneric categories. Computer analysis of large sets of characters in the hope of arriving at useful combinations have also been attempted by some workers.

3.2b *Biochemistry*: Most studies in this line involved the electrophoretic patterns of

the egg proteins, serological relationships between egg antigens as well as hemocytological studies. Of these, electrophoretic studies on enzymatic similarity are being increasingly used to trace genetic relationships among taxa showing close phenotypic resemblance. At the lower taxonomic levels, the more labile group II enzymes have been successfully employed to distinguish between species having identical anatomical phenotypes. However, the biochemical compounds fail to simplify taxonomic interpretation of disjunct groups of contemporary populations (Stephen 1973). The importance of comparative biochemistry in systematics lies principally in characterising homologues at the supraspecific levels.

4. Relevance of biosystematics in forest entomological research

Two aspects, viz the management of forestry important insect pests as well as conservation of ecologically important species are currently the main themes in forest entomological research (Collins and Morris 1985). With increasing demand for timber, forest plantations of a variety of indigenous as well as exotic tree species are being raised in extensive plantations. In addition to this, hybrids of several plant species are also being developed for possible resistance against insect or disease problems as well as for boosting productivity. Such large scale afforestation programmes involving the introduction of new germplasm may affect the biological attributes of organisms. Since the characteristics of an organism are to a great extent governed by climatic, edaphic or biotic factors, drastic changes in the ecosystem may also affect the biotic balance between various organisms. The recent epidemic of the bagworm, *Pteroma plagiophleps* on the introduced trees, *Albizia falcataria* as well as *Delonix regia* in Kerala is a striking example to this. This insect when first reported from southern India was only a pest of minor importance on *Tamarindus indica* and epidemics due to this insect have never been reported either on *T. indica* or any other native tree species. Biosystematic studies can play a major role in elucidating the factors leading to such pest outbreaks on forest plantations.

Other than the appearance of new pests, the development of new host races has been a phenomenon observed in many Lepidoptera. The host races behave differently from the natural populations of the species and establishment of the exact identity is very often required since modern pest management strategies are highly species specific.

With the increasing interest on conservation of nature, studies on the forest ecosystem are becoming very important. Correct identification of species is fundamental to any ecological study. Lepidopterans being mostly phytophagous affect the distribution and abundance of vegetation in any habitat. Shapiro (1985) reports that feeding by an inflorescence feeding pierid (*Anthocharis sara stella*) destroyed between 41 and 49% of potential seed output of a crucifer in California. Information on the extent of variation in the wild condition will also be of indicative value in monitoring the effect of environmental changes on organisms, since the phenomenon like industrial melanism is known to produce strikingly different colour patterns in many lepidopterans.

Since the objective of a classification is to allow easy identification of the included taxa, the classificatory schemes need to be simple to operate. In the morphotaxonomy direct matching of species is still considered to be an accepted method of identi

fication, and this limits its application in dealing with intraspecific categories with comparatively less morphological distinction. Taxonomic ranking of such categories though not much needed for the conventional taxonomist, is needed for the applied researcher who is working with living organisms. Since biosystematics involve studies with the living organism, it can not form part of the conventional taxonomy and probably further refinement is necessary to make it more practicable for routine identification of Lepidoptera.

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Recent trends in the biosystematics of Entognathous Apterygota with special reference to Collembola

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Abstract. In recent years increasing number of studies have been undertaken involving modern techniques like DNA hybridization, analysis of enzymic and chromosomal polymorphism in conspecific populations, ecophysiology and behaviour in individual species etc with far reaching implications in the systematics of Entognathous Apterygota, particularly the Collembola. Both scanning and transmission electron microscopes have been used extensively for the study of surface structure, sense organs, spermatozoa and gut providing considerable insight into phylogenetic relationship of higher taxa as well as inter-relationships at the generic and lower taxonomic levels.

Keywords. Insect biosystematics; Collembola; Protura; Diplura.

1. Introduction

Collembola, Protura and Diplura are regular inhabitants of the soil of natural forests and grasslands as well as others having good plant cover. Collembola are numerically most abundant and faunistically most diverse of the 3 groups. They have been found to colonize not only almost any type of soil with sufficient moisture but also the bark of trees, moss and to some extent fresh water bodies and marine systems (Prabhoo 1970, 1971a, b, c). An ecological study of soil invertebrates could hardly be complete without due consideration being given to the 3 groups mentioned above. Naturally meaningful ecological investigations can be undertaken especially on community structure, species diversity and niche, only with a proper understanding of the biosystematics of the groups.

Ever since the group Collembola has been established in 1862 by Lubbock, only morphological characters were utilised in the systematics of this group. Morphological variations were observed in geographical isolates (Prabhoo 1967) and local populations (Prabhoo 1976). With many new species being added to the existing list annually and with the accumulation of interesting information on biology and ecology of Collembola, systematists started looking up on the species from different angles for their delimitation and for understanding their genesis and phylogenetic position. An early attempt in this direction was the ecological categorisation of Collembola based on the concept of life forms made by Gisin (1943), subsequently modified by Cassagnau (1961) and Christiansen (1964). These categorisations, mostly based on external features were found to be inadequate especially in the case of cave dwelling forms in which specific features of biology and ecophysiology were recommended for study (Thibaud and Vannier 1986). Further, considerable information has become available on the chromosomal and protein polymorphism, ultrastructure and behaviour of Collembola in the recent past. An assessment of the studies carried out in the past 25 years to improve the species concept, phylogenetic relationship at various levels of taxa as well as speciational mechanisms is attempted

here so that an integrated approach in the systematics of these groups would be possible in future.

2. Cross hybridization of human and collembolan DNA

A variety of techniques are now available for hybridization of any species of DNA or RNA using labelled probes (Southern 1975; Britten and Davidson 1985). Stable and complete hybridization of two homologous DNA strands is possible under very stringent conditions, while partially homologous strands will hybridize under low stringency conditions (Howley *et al* 1979). The latter technique would be especially useful to study the degree of sequence homology between distantly related DNA's.

Fodde *et al* (1986) employed the human haptoglobin gene (DNA segment) to isolate from the Collembola *Thaumanura ruffoi* the DNA sequence sharing high degree of homology with the former. The authors incubated filter immobilised single stranded DNA from different species of animals, including *T. ruffoi*, with labelled Haptoglobin complementary DNA (Hp cDNA) at various stringencies. Autoradiographs showed that at the lowest stringency (washing at 42°C) a distinct band (6 kb) appeared with *T. ruffoi* genomic DNA as well as with those of some other species, with considerable amount of background formed by nonspecific hybridization. In two further sets of experiments the stringency was increased by increasing the temperature of washing to 55° and 62°C. This resulted in progressive disappearance of all nonspecific background in the autoradiographs. The band exhibited by *T. ruffoi* genome was found to be stable even at the highest stringency.

The experiments confirmed the presence of human haptoglobin like sequence in the Collembolan *T. ruffoi*. It is possible to use additional DNA probes to identify other base sequences in this insect. By isolating and cloning such fragments of established base sequence it is hoped to employ them in DNA hybridization studies which would lead to a better understanding of the systematic position and interrelationship of over 1500 species of Collembola. Fodde *et al* (1986) suggest that nucleotide sequence comparison and restriction fragment length polymorphism analysis used by other workers (Helm-Bychowski and Wilson 1986; Sibley and Ahlquist 1986) to reconstruct bird phylogeny could be employed once *T. ruffoi* probes become available. This would be a new approach for the study of systematics and genetics of Collembola.

3. Use of electrophoresis in Collembolan population analysis

Advances in electrophoretic techniques now permit one to analyse multiple fractions of proteins in a sample by staining methods and show that to a great extent such variations (allozymic) are inherited in a Mendelian fashion. Large number of proteins in the same species can thus be studied for understanding genic variability and population structure (Goettlibe 1971). The rationale behind electrophoresis of proteins (enzymes) is not difficult to understand (Avise 1974). Proteins migrate through an electric field according to the net charge and also depending upon the molecular weight. Those migrating to different distances usually differ at least by one amino acid residue. The relationship between amino acid sequence in proteins and DNA base sequence is well established. Hence electrophoretic mobility of proteins provides indirect information about DNA.

An attempt was made by Dallai *et al* (1983) to measure the genetic distance among ian populations of two Neanurid species *Bilobella aurantiaca* and *T. ruffoi.* Only ymes that could be separated electrophoretically were selected for the study, 4 in first and 15 in the second species. One of the populations was considered as the trol for measuring genetic distance among local populations. They found that *ruffoi* was monomorphic at 6 of the 15 loci studied while the remaining 9 loci were ymorphic for two or more alleles. Low genetic distance [calculated employing i's (1972) method], was noted between Siena and Barbione populations which re closely situated geographically. High level of genetic distance was evident ween Siena/Barbione and Naples population which was comparable to that ween well differentiated species which were reproductively isolated (Ayala 1975; ise 1974). In yet another study of *T. ruffoi* populations from 3 more localities and *Lathriopyga longiseta* (Fanciulli *et al* 1986a, b) it was found that the coefficient of netic identity and genetic distance indicated that the populations had reached a h degree of genetic differentiation. According to the above workers if one nsiders that the value of genetic divergence permitting to attain reproductive lation without morphological differentiation is the same as that in other ganisms (Ayala 1983), then it is only proper to consider that the different pulations at least of *T. ruffoi* were evolving independently and should probably be nsidered as belonging to different taxa. In *L. longiseta* a cline was suspected. It is dent from the above studies that species of Neanurinae with limited powers of persal could form isolated small populations wherein several factors including ft could play an important role in producing genetic divergence among pulations.

Polytene chromosome in Collembola

lytene chromosomes so characteristic of salivary gland cells and few other tissues larval Diptera, played an important role in the understanding of the systematics d evolution of many dipteran families. In Collembola such chromosomes were covered (Prabhoo 1961) in a neanurid species in the adult salivary gland cells. sic configuration of the salivary gland complex and polytene chromosomes of *aurantiaca* has been described by Cassagnau (1968a). Considerable diversity has en noted in the gross morphology and cytology of the salivary glands, features that pear to be characteristic at the generic level, but in some cases at the species level ee 1985a). Species with polytene chromosomes always have stylet like mouth parts assagnau 1968b) though the converse is not true. Thus pseudachorutinae and putanurinae with stylet like mouth parts do not have polytene chromosomes.

Population variations in polytene chromosome structure

atures characteristic of the polytene chromosomes are the puffs (Balbiani rings) d the heterochromatin of two types—α- and β-heterochromatin of which the latter ess condensed and takes up less intense stain but generally occupies a major part of chromosome. Somatic pairing and a diffuse chromocenter are characteristically sent in the polytene chromosomes of Collembola.

3.3 *Population variation in polytene chromosome structure*

Several workers (Cassagnau 1976; Dalens 1976, 1977, 1979; Dallai 1979; D 1982) have studied chromosomal polymorphism in *B. aurantiaca.* understanding of the phenomenon proved difficult because of the difficulty i homologous sites in the chromosomes. In a more recent study of the abov Deharveng and Lee (1984) devised a system of nomenclature of the heteroc bands based on their location in 32 sites of the 7 chromosomes (n = 7) in t Baume population (France). Comparison of this population with Gredos pc (Spain) indicated a marked stabilisation of the karyotype with i heterochromatin load, a feature noted by Dallai (1979) in the Italian popu the species. Further heterochromatic load was more important in the (north Italy, southern France) and Atlantic (northern Portugal) races than living in more xeric and warmer environments (Spain, southern Portugal, Italy, Morocco) of the distribution of the species. It has already been show that heterochromatic load increased in *Deutonura monticola* with the enviro situation becoming unfavourable (Cassagnau 1974; Deharveng 1982).

3.4 *Species differences in polytene chromosomes*

Haploid number of chromosomes in several species of *Bilobella* was found Hardly any karyotypic difference could be made out in the dot chron characteristic of Collembola. On the other hand based on the band sequen polytene chromosomes and other features like the relative length chromosomal arms and taking *B. aurantiaca* as the reference species, Cassag (1985) categorized the 7 chromosomes in 7 species of *Bilobella* into 11 ca Each species possessed a more or less unique combination of 11 categ chromosomes such that the 7 species of *Bilobella*, with relatively uniform features, fell into 3 groups, members of a group being more closely related other than to members of other groups. The 3 groups were *B. proxima* and *B. n B. massoudi* and *B. zekoi* and lastly *B. albanica, B. aurantiaca* and *B. digitata.* karyotypic studies could thus help understanding interrelationship of co species.

4. Electron microscopic studies

4.1 *Cuticular structure in Collembola*

Two types of granules, primary or single elementary granule triangular in fo secondary granule formed by the association of certain primary granules wer nized by Massoud (1969) in the epicuticle of Collembola. This study stimulate work on many species (Lawrence and Massoud 1973; Lawrence 1975; Dall In Entomobryidae one finds the primitive pattern of triangular primary arranged hexagonally. This pattern is noted in other families in restricted re the body like head, antennae, legs, furca etc. Isotomidae basically have connected quadrangular granules. The cuticular pattern is more or less con

many genera of Hypogastruridae, Neanuridae and Onychiuridae (Massoud 1969). It is possible that the cuticular pattern is intimately related to ecological factors (Dallai 1977). If so it will not help much in the understanding of the phylogeny. On the other hand the cuticular structure does not change in the course of development and hence it can be of considerable value in classification and discrimination of taxa. Cuticle morphology, however, has the same pattern in members of Entomobryidae, Cyphoderidae, Tomoceridae and Oncopoduridae. On the other hand in the tribe Sminthurini almost each genus has a peculiar pattern (Dallai 1977). Cuticular pattern of *Sminthurinus echinatus*, *S. marginatus* and *S. bremondi* is very different from that of other species of the genus so that the creation of the genus *Caprainea* for accommodating the above 3 species on the basis of other features like the presence of trochanteral organs and postantennal sensory hair seems justified (Dallai 1977).

4.2 *Postantennal organ of Collembola*

The postantennal organ situated just behind the antennae in the lower families of Arthropleona viz Hypogastruridae, Onychiuridae and Isotomidae. Light microscope studies revealed a limited diversity of structure of these organs and the characters were widely used in the systematics of the groups. The organ is supposed to be sensory in function. Scanning electron microscopic (SEM) studies (Dallai 1971) showed that the cuticle is continuous and undulated in Hypogastruridae but is perforated in Onychiuridae and Isotomidae. Further TEM studies of the organ in *Anurida maritima* indicated that it is olfactory in function (Dallai 1971).

4.3 *Pseudoculi in Protura*

Protura lack antennae, but in their place are found a pair of curious structures called pseudoculi, certain features of which are utilised in the systematics of the group. SEM and TEM studies (Bedini and Tongiorgi 1971; Yin *et al* 1986) confirmed the sensory nature of these organs and besides indicated that there are mainly two types of pseudoculi. The first type has striations and furrows on the surface and is the most common type found in *Eosentomon*, *Neocondellum*, *Hesperentomon*, *Acerentomon*, *Acerentulus*, *Gracilentulus* and *Baculentulus*. There are pore canals at the bottom of the furrows connected directly to the dendrites of 3 large sensory cells. In the second type the surface shows an orderly array of pores, each with a tubule and there are 6–8 sensory cells. This type is found in *Fujientomon* and *Sinentomon*, throwing light on the phylogenetic position of the former.

4.4 *Ultrastructure of spermatozoa*

Ultrastructure of spermatozoa has received special attention in the study of phylogeny of Arthropoda (Baccetti 1979). In insects the primitive 9+2 filaments in the axoneme (composed of tubulin and dynein) is found only in Collembola and some Diplura. In Collembola the sperm is encysted (Dallai 1969) but motile, and in Japygidae (Diplura) the condition is more or less similar but the sperm has an extra set of microtubules in the mitochondrial compartment. This latter model has

apparently paved the way for the evolution of the classical insectan sperm with axonemal complex of 9+9+2 filaments, found in Campodeidae (Diplura), Thysanura and Pterygota. The proturan sperm is not in the direct line of evolution of the insectan sperm being specialised and evolved with loss of dynein and hence has become immotile with the axoneme pattern 12+0 in *Acerentulus* and 14+0 in *Acerentomon* and finally becoming aflagellate and disc shaped in Eosentomidae by the loss of acrosome and axonemal complex and in *Sinentomon* it has become aflagellate and spherical. These studies throw light on the phylogenetic position of Protura and relationship within the group. Based on sperm ultrastructure it would be difficult to agree with the classical view that Eosentomidae are more primitive than Acerentomidae.

4.5 *Gut ultrastructure and phylogenetic interrelationship of Entognathous groups*

Recent work on the ultrastructure of the gut of Entognathous Apterygota has thrown some light on the interrelationship within the group (Dallai 1976; Dallai and Callani 1979; Dallai and Burroni 1982). Comparative study of the ultrastructure of the midgut and hind gut indicates that the Protura and Collembola are aberrant groups, the former due to the possession of a peculiar pyloric chamber with specialised cells bearing long microvilli behind the sphincter and the latter because of the possession of a peculiar pyloric ring and connecting cells in the pyloric region just behind the midgut. Diplura possess some features of one or the other of the above two groups. Thus *Campodea* has similarities with Protura in possessing the Malpighian papillae but there is no pyloric chamber while *Japyx* has similarities with Collembola in having the connecting cells but there is no pyloric sphincter. Nor is there any trace of Malpighian papillae. Tuxen (1972) and Kristensen (1975) consider Protura and Collembola as sister groups of Diplura. Probably the ultrastructure of the gut lends support to this view.

5. Cladistic analysis of taxonomic characters

Cladistics has its origin in the Henning School (Henning 1966) which recognizes relationships among taxa at all levels on the basis of dichotomous branching (Klados = branch). In this system the characters are considered to be mainly of two types, those inherited from the ancestor are plesiomorphous and those that are special ones are apomorphous. Shared apomorphous characters are synapomorphous. Relationship among taxa is sought to be understood only on this basis.

Lee (1985b) tried to establish phylogenetic relationship at the subfamily level within Neanuridae by cladistic analysis using character weighted (Schaeffer *et al* 1972) and character unweighted (Kluge and Farris 1969) methods. Twenty characters were used for the purpose and the family Hypogastruridae was taken as the ancestral out group for character comparison and for computing branching sequence. Distance of various subfamilies of Neanuridae from the ancestor is then determined (Kluge and Farris 1969; Wiley 1981) and the cladograms obtained following the two methods although looked different, the position and arrangement of the taxa with respect to the ancestor and in relation to each other was very similar except for the fact that the position of the Neanurinae and Caputanurinae was reversed. It appears

that the above methods could be employed fruitfully for understanding the phylogeny of especially higher categories of taxa.

6. Behavioural studies and systematics of Collembola

Behavioural studies have contributed much to the clarification of problems in systematics. As behaviour constitutes most diverse of phenotypic characters and this feature is perhaps more strongly selected than morphology or physiology it can provide important criteria for characterising taxa at different levels.

Complicated behaviour patterns related to reproduction are shown by different groups of animals including insects. One may feel that the degree of complication is related to the evolutionary status of the groups determined on the basis of other characteristics chiefly morphological. Reproductive behaviour in Collembola is particularly associated with sperm transfer (Schaller 1971). These insects do not have external genitalia and hence the sperm transfer is shown to be indirect through a sperm packet or spermatophore. Mechanism of spermatophore production appears to be complicated as is shown in the case of *Orchesella cincta* by Doring (1986). Two ways of spermatophore transfer is recognized. The first one is effected without mating, considered as primitive and is noted in almost all Arthropleona and a few Symphypleona studied so far. Reviewing the mechanism of sperm transfer in Burletiellinae Betsch (1974) suggested that the subfamily could be divided into two. The Bourletiellinae should comprise of the genera *Bourletiella*, *Deuterosminthurus*, *Heterosminthurus* and others in which the sperm transfer occurs with mating (Bretfeld 1971). In the other group of genera *Bourletiellitas*, *Anjavidiella* and *Vatomadiella* as well as *Parabourletiella* and *Paulianitas* (Betsch 1980), all confined to Madagascar, the males deposit the spermatophores at random and females pick them up without mating. This group according to Betsch (1974, 1980) should be recognised as a separate subfamily Parabourletiellinae. Bretfeld's (1971, 1973, 1976a, b) studies showed how reproductive behaviour related to sperm transfer in the 4 species of *Heterosminthurus*, viz *novemlineatus*, *insignis*, *lineatus* and *clviger* is specific and can serve as diagnostic features.

A second type of behaviour is concerned with protection against dessication and is revealed as behaviour in nest construction or anhydrobiosis. Information on the nest building behaviour in a few species of Collembola is given by Poinsot (1971). Consideration of these behaviour patterns has helped distinguishing species of some genera like *Folsomides* in which overlapping of characters due to variability of certain morphological features like ocelli create a certain degree of uncertainty in determination at the species level. Poinsot-Balaguer and Barra (1982) used morphological features in combination with behaviour to distinguish *Folsomides americanus*, *F. angularis*, *F. deserticola*, *F. sexophthalma*, *F. novacerradensis* and *F. corsicus* and to support the contention that *F. deserticola* is a synonym of *F. sexophthalma*.

7. Conclusion

It appears from the above that there is an urgent need to undertake study of population phenomena in Entognathous Apterygota. This will help understanding the

mechanism of speciation on the one hand and elucidation of ecological importance of the species on the other leading to discovery of species that indicate specific ecological conditions. Sibling species are also likely to be brought to light. Evidently these objectives can be realised only by using modern methods of research. Cytological studies of polytene chromosomes can be made only in some genera of Neauuridae which possess polytene chromosomes. On the other hand study of enzyme polymorphism employing sensitive electrophoretic techniques can be undertaken in all groups wherever necessary. Similarly the study of behaviour and ecophysiology of all important species should be undertaken along with electron microscopic studies. An integrated approach to the study of taxa appears extremely desirable.

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Biosystematics of *Culex vishnui* and *Culex pseudovishnui* based on ecobehavioural pattern

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Abstract. *Culex vishnui* group consists of 3 species of mosquitoes viz *Culex vishnui*, *Culex pseudovishnui* and *Culex tritaeniorhynchus*. It was found that out of 16 species of mosquitoes collected in 3 years from two different biotopes, 8·28 and 24·89% of *Culex vishnui* mosquitoes were collected from human habitations and cowsheds respectively. The corresponding data of *Culex pseudovishnui* were 1·80 and 5·66% respectively. Out of 12274 mosquitoes belonging to 17 species collected off man-baits in one year, *Culex vishnui* constituted 34·6% while the latter species encountered 9·04%. When the collection was made by placing man-baits and cow-baits side by side, the attraction ratio (man : cow) of *Culex vishnui* was found to be 1 : 1·9 and that of *Culex pseudovishnui* was 1 : 2·6. Bloodmeal analysis of these two species of mosquitoes revealed that *Culex vishnui* was more inclined to human blood (21·5%) than that of *Culex pseudovishnui* (16·14%). *Culex vishnui* and *Culex pseudovishnui* were both susceptible to DDT, dieldrin and malathion, but the LT_{50} value was slightly higher in the former species than the latter. *Culex vishnui* utilised its breeding spots like paddy fields, ponds, ditches and burrow-pits more effectively in almost all the months of a year than *Culex pseudovishnui*. The density of the larvae of *Culex pseudovishnui* was found to be lower than that of *Culex vishnui*. Japanese encephalitis virus was isolated only from *Culex vishnui* in West Bengal.

Keywords. Biosystematics; *Culex vishnui*; *Culex pseudovishnui*; ecobehavioural pattern.

1. Introduction

The activity of Japanese encephalitis (JE) in India is known to occur in the southern part since the 50's (NIV 1980). During the period 1955–56, altogether 17 strains of JE virus have been isolated from *Culex vishnui* group in south India (Hati 1981). However, until 1969, the taxonomic status of *Cx. vishnui* group was not very clear. It was Reuben (1969) who first demonstrated that this group consists actually of 3 species viz *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. tritaeniorhynchus*. Later on, several investigators (Rajagopalan and Panicker 1978; Soman *et al* 1980; Mishra *et al* 1984) reported that in the rural areas of India *Cx. vishnui* group is being represented by 3 distinct species as mentioned by Reuben. *Cx. vishnui* and *Cx. pseudovishnui* are prevalent in the rural areas of West Bengal (Soman *et al* 1976; Mahadev *et al* 1978). Uptil now *Cx. vishnui* has been the only member of the *Cx. vishnui* group, incriminated as the vector of JE in the state of West Bengal (Chakraborty *et al* 1980). The present study was conducted in order to find out whether the ecobehavioural pattern of *Cx. vishnui* and *Cx. pseudovishnui* also corroborate the idea of Reuben (1969).

2. Materials and methods

2.1 *Study area*

A typical village, named Nudipur in the district of Burdwan, West Bengal state, 80 km from Calcutta was selected as the central field station. Another village, named

Korea, in the same district 15 km from Nudipur village was selected only for bait preference study. No insecticide had been sprayed in those two villages for the last 20 years.

2.2 *Study period*

The period of study extended from December 1980 to November 1983.

2.3 *Collection of mosquitoes*

Aspirators and battery operated torches were used for collection of adult mosquitoes. Mosquitoes were collected thrice a week during one hour in the morning (0600 AM–0700 AM) from cow sheds and human habitations. For the collection of mosquitoes, the methods of Holstein (1954) have been adopted with suitable modifications.

Mosquitoes (female) were collected off man-baits overnight from 1800–0600 h weekly once for one year following the method of Hati *et al* (1981) with necessary modifications.

Mosquitoes (female) were collected from man-baits and animal baits placed side by side from 1800–0600 h twice a month throughout the year. The method of Hati *et al* (1981) was employed for this comparative study of bait preference with necessary modifications.

2.4 *Bloodmeal analysis*

Fully fed females of *Cx. vishnui* and *Cx. pseudovishnui*, collected from human habitations and cow sheds, were selected for bloodmeal analysis so that no difficulty would be encountered in conducting the test. The stomach contents of the females were dissected with a pair of needles and the abdominal contents were squeezed on to the filter paper. The test was performed in wells prepared on glass slide with agar gel by modified Ouchterlony gel diffusion method (Ouchterlony and Nilson 1973) as adapted by Collins *et al* (1983). The antisera used in the analysis were prepared by the laboratory of the Serologist and Chemical Examiner to the Government of India, Calcutta. The samples were run against antihuman, antibovine, antiporcine and antiavian sera.

2.5 *Insecticide susceptibility tests*

Insecticide susceptibility tests were carried out (December 1982 to November 1983) using WHO kits, following WHO (1970) techniques.

2.6 *Collection of mosquito larvae*

Out of 3 methods of collection of mosquito larvae, viz dipping, netting and pipetting advocated by WHO (1975), the first method was adopted in the present study. From the fields, ponds, ditches and burrowpits, collection of larvae was attempted twice a month for one year.

3. Results

3.1 *Wild caught*

Altogether 34155 mosquitoes belonging to 16 species were collected from two different biotopes in 3 years, of which *Cx. vishnui* topped the list (33·17%) while *Cx. pseudovishnui* occupied fifth position, which constituted only 7·46%; 8·28 and 24·89% of *Cx. vishnui* mosquitoes were encountered in human habitations and cowsheds respectively. The corresponding data of *Cx. pseudovishnui* were 1·80 and 5·66% respectively (table 1). The per man-hour collection of *Cx. vishnui* was found to be highest in the rainy season (15·59) and lowest during the winter (10·34). The highest and lowest per man-hour collections of *Cx. pseudovishnui* were obtained in the rainy season (3·73) and summer (2·03) respectively. A strain of JE virus was isolated from a pool of *Cx. vishnui* mosquitoes, collected from human habitations in September, 1982 (table 1) (figure 1).

Table 1. Comparative study of ecobehavioural pattern of *Cx. vishnui* and *Cx. pseudovishnui* using several set parameters.

	Cx. vishnui	*Cx. pseudovishnui*
Wild caught (%)		
Human habitation	8·28	1·80
Cowshed	24·89	5·66
Total	33·17	7·46
Per man-hour collection		
Summer	13·40	2·03
Rainy season	15·59	3·73
Winter	10·34	3·08
Collected off man-bait (%)	34·6	9·04
Collected off man and cow bait (%)	27·90	8·84
Man : Cow ratio	1 : 1·9	1 : 2·6
Bloodmeal analysis (% positive for human blood)		
Human habitation	15·9	12·7
Cowshed	5·5	3·5
Total	21·5	16·14
Insecticide susceptibility test LT_{50} values (min)		
DDT	37·05	32·32
Dieldrin	25·80	23·55
Malathion	7·25	6·25
Larval density (larvae/dip)		
Fields	1·93	0·62
Ponds	0·51	0·16
Ditches	0·25	0·12
Burrowpits	0·31	0·24
Detection of JE virus	+	−

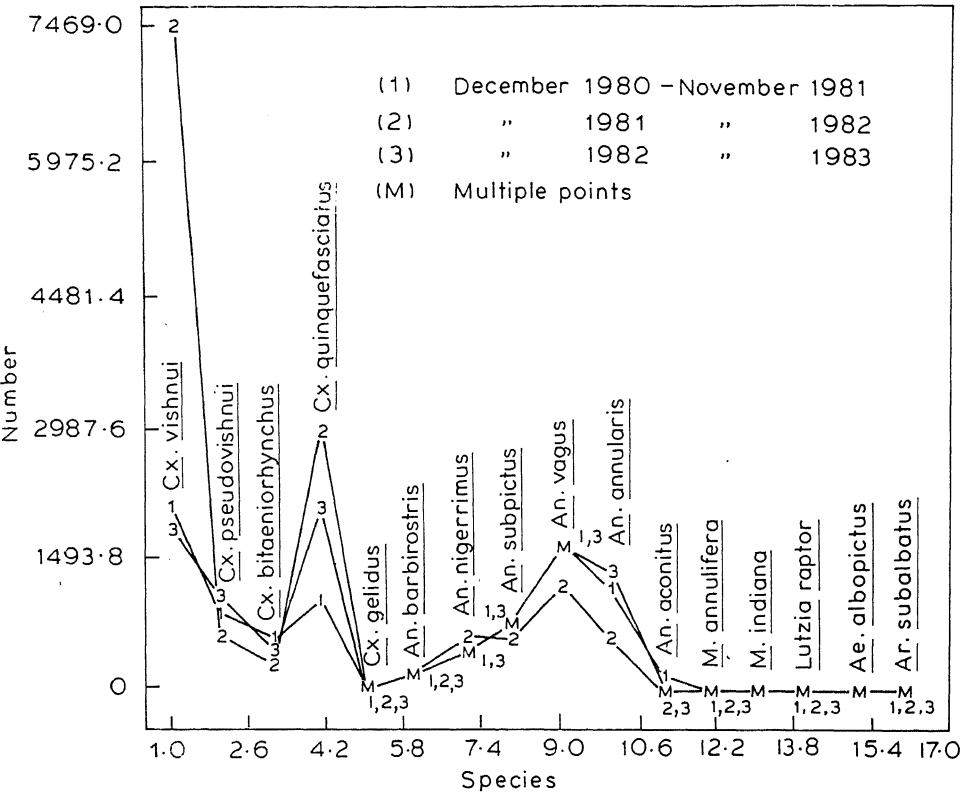

Figure 1. Composition of mosquito fauna of Nudipur village, Burdwan district in different biotopes in 3 consecutive years.

3.2 *Man-bait*

As a whole, 12274 mosquitoes (female) comprising of 17 species were collected off man-baits during the 12 months study period. Altogether 4247 *Cx. vishnui* and 1110 *Cx. pseudovishnui* were collected, comprising of 34·6 and 9·04% of the total respectively (table 1).

3.3 *Bait preference*

A total of 20375 mosquitoes (female) belonging to 12 species were attracted to human and cow-baits. *Cx. vishnui* and *Cx. pseudovishnui* occupied the first (27·90%) and the fifth (8·84%) positions respectively. The attraction ratio (man:cow) of *Cx. vishnui* and *Cx. pseudovishnui* was 1:1·9 and 1:2·6 respectively (table 1) (figure 2).

3.4 *Bloodmeal analysis*

Out of 563 *Cx. vishnui* mosquitoes tested, 121 (21·5%) were found to be positive for human blood (table 1), 357 (63·4%) for bovine blood, 59 (10·5%) for porcine blood

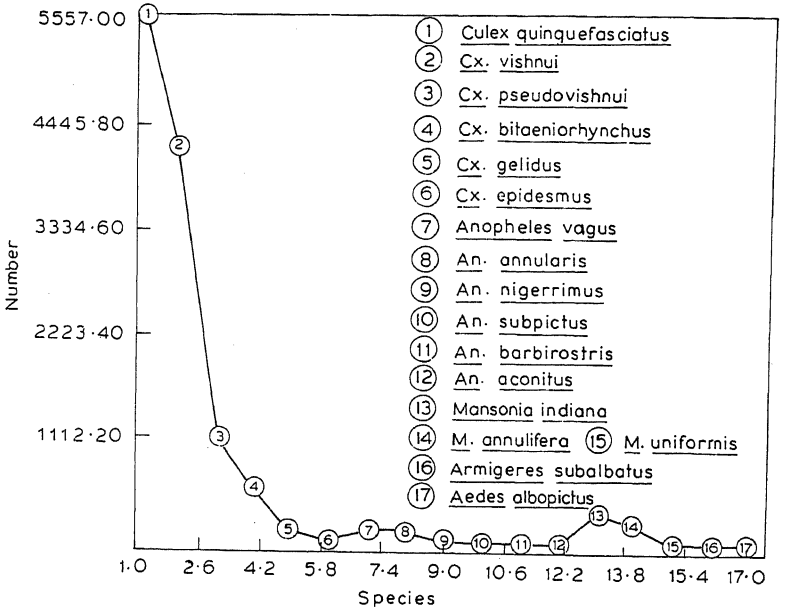

Figure 2. Composition of 17 species of mosquitoes caught off human baits (December 1980–November 1981).

and 26 (4·6%) for avian blood. The corresponding figures for *Cx. pseudovishnui* were 93 (16·14%), 404 (70·1%), 60 (10·4%) and 19 (3·3%) respectively out of 576 tested.

3.5 *Insecticide susceptibility test*

Altogether 100 wild caught *Cx. vishnui* mosquitoes were taken for each insecticide test in each exposure. In 15 min, 30 min, 1 h and 2 h exposure to DDT (4%), the mortality of the species was 8, 42, 78 and 100 respectively. The mortality of *Cx. vishnui* in 15 min, 30 min and 1 h exposure to dieldrin (0·4%) was 14, 64 and 100 respectively. It was observed that in a 15 min exposure to malathion (5%), 100% *Cx. vishnui* were killed within 24 h. With the same exposure period for each insecticide, the mortality figures of *Cx. pseudovishnui* were 12, 46, 90 and 100 in DDT; 18, 74 and 100 in dieldrin and 38, 82 and 100 in malathion respectively. LT_{50} in min of adult *Cx. vishnui* to DDT, dieldrin and malathion were 37·05, 25·80 and 7·25 respectively. The corresponding data for *Cx. pseudovishnui* were 32·32, 23·55 and 6·25 respectively (table 1) (figure 3).

3.6 *Breeding sites and larval density*

Out of 6 species of mosquito larvae viz *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus*, *An. barbirostris*, *An. nigerrimus* and *An. subpictus* collected from the fields, ponds, ditches and burrow-pits, *Cx. vishnui* larvae were obtained in all the 4 breeding sites in almost all the months throughout the seasons. While the monthwise distribution of *Cx. pseudovishnui* larvae was found to be limited (present for only 7 months

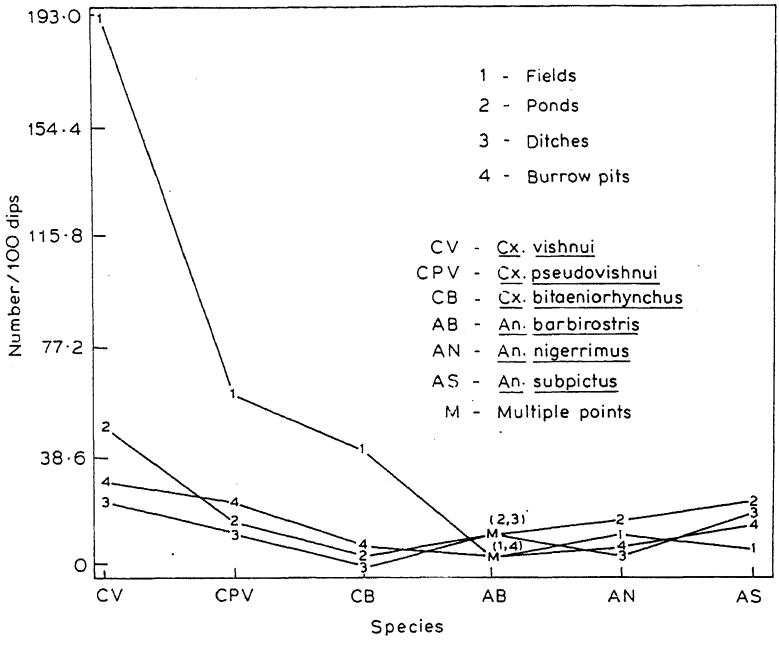

Figure 3. Total collection of different species of mosquito larvae from fields, ponds, ditches and burrowpits (April 1982–March 1983).

in the fields, 6 months in ditches and ponds and 5 months in burrowpits). Yearly collection of *Cx. vishnui* larvae per dip in the fields, ponds, ditches and burrowpits were 1·93, 0·51, 0·25 and 0·31 respectively. The corresponding data for *Cx. pseudovishnui* were 0·62, 0·16, 0·12 and 0·24 respectively (table 1).

4. Discussion

Ecological data have always been involved in taxonomy in a broadway and are also now increasingly important and precise. Species at any one time and place are ecologically incompatible (Simpson 1969). Mayr (1958) demonstrated and adequately illustrated the taxonomic usefulness of behavioural data.

Cx. vishnui mosquitoes constituted 8·28 and 24·89% of the total catch in human habitations and cowsheds respectively whereas *Cx. pseudovishnui* encountered in those habitats was only 1·80% and 5·66% respectively. The data show that in each biotope, density of *Cx. vishnui* population was significantly higher than *Cx. pseudovishnui* in this area. The per man-hour collection of *Cx. vishnui* was found to be the highest in rainy season and lowest in winter, while *Cx. pseudovishnui* was obtained in greater numbers in the rainy season and lowest collection was evident in summer. This finding suggests that *Cx. vishnui* and *Cx. pseudovishnui* populations differ not only quantitatively with each other but also seasonally; although in the rainy season a higher catch was recorded for both the species. Mosquito population, in general, find the rainy season favourable for breeding owing to the availability of more natural breeding grounds.

Significantly more *Cx. vishnui* mosquitoes were attracted to human beings than *Cx. pseudovishnui*. The density ratio of these two culicine species was found to be 1 : 3·8 (*Cx. pseudovishnui* : *Cx. vishnui*).

When animal baits (cow) were placed near human baits, a significantly greater number of *Cx. vishnui* mosquitoes were caught off man baits in comparison to *Cx. pseudovishnui*. The attraction ratio (man : cow) of *Cx. vishnui* and *Cx. pseudovishnui* was found to be 1 : 1·9 and 1 : 2·6 respectively. This observation suggests that the latter species have high predilection for animal baits than the former. But, in both the situations (i.e. in man-bait and bait preference studies) *Cx. vishnui* was significantly found to be attracted to man-baits than its counterpart, *Cx. pseudovishnui*. This observation agrees with the findings of Self *et al* (1975) in Indonesia.

Bloodmeal analysis of these two species of mosquitoes has also revealed that *Cx. vishnui* was more inclined to human blood than *Cx. pseudovishnui*.

The criteria for interpreting the results on the susceptibility status against DDT are arbitrarily adopted for explaining the results of single susceptibility test with mosquito populations collected in the field as mentioned by Ismail and Phinichpongse (1980) and they are basically on those recommended by Davidson and Zahar (1973) with some modifications for field application in West Bengal. Using the above criteria it was found that *Cx. vishnui* and *Cx. pseudovishnui* were both susceptible to DDT, dieldrin and malathion; but differed in their LT_{50} values. The LT_{50} value was found to be higher in *Cx. vishnui* than in *Cx. pseudovishnui*.

Cx. vishnui could effectively utilise the different breeding sites such as paddy fields, ponds, ditches and burrowpits in almost all the months throughout the seasons, while the monthwise distribution of *Cx. pseudovishnui* was found to be limited. In all the 4 biotopes the former species topped the list as far as the density (larvae/dip) was concerned. In the fields and burrowpits *Cx. pseudovishnui* occupied the second position while in the ponds and ditches the position was found to be third and fourth respectively. The largest number of *Cx. vishnui* larvae was evident in the fields than in the other 3 breeding sites. Similar observation was made by Reuben (1971) in south India. Uptil now *Cx. vishnui* has been the only member of the *Cx. vishnui* group incriminated as the vector of JE in the state of West Bengal.

All the observations stated above on ecobehavioural pattern of the two culicine species, suggest that *Cx. vishnui* and *Cx. pseudovishnui* are ecologically incompatible in this area and this finding reinforces the idea of Reuben (1969).

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Biosystematic studies of south Indian Leptophlebiidae and Heptageniidae in relation to egg ultrastructure and phylogenetic interpretations

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Abstract. Previous work on the importance of egg ultrastructure to the taxonomic and phylogenetic studies of Ephemeroptera with special emphasis on the two schistonotan families, Leptophlebiidae and Heptageniidae is reviewed. The chorionic sculpturings, the types of micropyles and attachment structures of the eggs of south Indian mayfly species belonging to 8 genera of Leptophlebiidae and 3 genera of Heptageniidae have been studied through scanning electron microscope.

The present study confirms the earlier observations that the Leptophlebiidae are most certainly of enough distant relationship with the Heptageniidae to warrent inclusion in separate superfamilies viz Leptophlebioidea and Baetoidea. Whereas the structural modifications on the eggs are significant in contributing to the taxonomy of both the families at the generic level, submicroscopic examinations of chorionic structures provide data for the separation of different species and species complexes in Leptophlebiidae.

Keywords. Biosystematics; Leptophlebiidae; Heptageniidae; egg ultrastructure; phylogeny.

1. Introduction

Biosystematic studies of the Ephemeroptera have gained much significance in recent years because of their importance as fish food, of pollution indication in freshwater ecosystems and in being ideal objects for biogeographic analysis due to their conservative dispersal habits. This group of insects is best suited for integrated approach to phylogeny, classification and biogeography due to its compact size enabling systematic studies on a global perspective. Furthermore, systematic conclusions can be arrived at through the study of a wide range of characters from different sources which include exoskeletal, soft anatomical, physiological and behavioural data from imaginal, nymphal and egg stages (Mc Cafferty and Edmunds 1979). The Ephemeroptera have various life history stages that have semi-independent genetic control, separate selection pressures and hence different rates of divergence. These differential rates of evolution in various stages are a powerful tool in the reconstruction of the sequence of branching in phylogeny. Differential rates of semi-independent or independent character systems within a life history stage can provide equally powerful data (Edmunds 1975). However, interpretations become rather difficult due to niche specialization, niche overlap and parallelisms. In such intriguing situations, electrophoretic (Kownacki and Starmach 1984; Matha and Sula 1984; Zurwerra *et al* 1984), karyological (Kiauta and Mol 1977) and egg ultrastructural (Koss 1968; Koss and Edmunds 1974) studies assume crucial importance as valuable tools in resolving enigmatic generic and species complexes.

Through a study of the ultrastructure of approximately 100 of the known mayfly genera, Koss (1973) made it evident that this group of insects can provide valuable data for taxonomic and phylogenetic studies of the order. One very important and outstanding feature of the mayfly reproductive cycle makes the egg stage ideally

suited for systematic studies. Unlike most insect groups, mayfly eggs mature during the nymphal or immature part of the life cycle, and therefore, eggs can be dissected from mature, black wing-padded nymphs for study. Also, those eggs may be compared to eggs dissected from adult specimens, and this procedure can be very helpful when trying to associate nymphs with adults when rearing is not possible and one of these stages is undescribed. The eggs possess morphological characters which are far more dependable than the body colouration of the adult.

Almost unique among mayflies is the fact that most adult females lay their eggs freely on the surface of the water rather than personally attaching them to any fixed objects. Because of the egg laying behaviour of the adult mayfly and the environment to which the eggs are released, these eggs are quite strongly subjected to the pressure of natural selection. These selection pressures have caused the evolution of a great variety of external features on ephemeroptera eggs to contribute valuable information for phylogenetic interpretations of the order. These features include, the chorionic sculpturings, the types of micropyles and attachment structures. The egg ultrastructural data provide for further testing of the numerous hypotheses involved in proposed phylogenies, and suggests revision of some of the present concepts of ephemeropteran phylogeny.

An attempt has been made to review the previous work on the importance of egg ultrastructure to the taxonomic and phylogenetic studies of the order with special emphasis on the two schistonotan families, Leptophlebiidae and Heptageniidae and to examine the importance of this study in providing valuable data for the separation of generic and species complexes in the light of the scanning electron microscopic study of the eggs of south Indian mayfly species belonging to 8 genera of Leptophlebiidae and 3 genera of Heptageniidae.

2. Perspectives and overview

Pioneering studies of importance of the eggs to insect taxonomy were made by Onsager and Mulkern (1963) for Orthoptera; Ross and Horsfall (1965) for Culicidae; Knight *et al* (1965a, b) for Plecoptera; Degrange (1960), Koss (1968) and Koss and Edmunds (1974) for Ephemeroptera; and Southwood (1956) for Heteroptera. Recently, numerous scanning electron microscope (SEM) studies have been performed on insect eggs (Hinton 1981). Most of these works revealed diagnostic characteristics useful in the assessment of phylogenetic relationships.

Previous SEM studies of the eggs of the Ephemeroptera have included Baetidae (Kopelke and Müller-Liebenau 1981a, b, 1982), Caenidae (Kopelke 1980; Malzacher 1982), Heptageniidae (Flowers 1980a, b; Kopelke 1980; Landa and Soldan 1982), Leptophlebiidae (Kopelke 1980; Pescador and Peters 1982; Gaino and Mazzini 1984; Mazzini and Gaino 1985; Towns 1983; Towns and Peters 1978, 1979a, b) and Tricorythidae (Kopelke 1980). These studies have revealed features of the chorionic sculpturing, micropyle and attachment structures that can be useful in understanding the phylogeny of Ephemeroptera.

Koss and Edmunds (1974) studied the eggs of representative genera of all families of Ephemeroptera except the Palingeniidae and found out that the archetypical Ephemeroptera egg is considered to have been round, with a smooth chorion, nonfibrous adhesive layer, funnelform micropyle, and a suprachorionic sperm guide.

Subsequent evolution resulted in several different micropyles and many different chorionic sculpturings and attachment structures. The ultrastructural features of the eggs of the superfamilies of Ephemeroptera are given in table 1. This is apparently in conformity with the phylogeny (figure 1) of the extant superfamilies and suborders of Ephemeroptera as suggested by Mc Cafferty and Edmunds (1979). The evidence from the study of Koss and Edmunds (1974) has suggested that within the

Table 1. Ultrastructural features of the eggs of superfamilies of Ephemeroptera.

Superfamily	Attachment structures	Micropyle (Chorionic sculpturing—variable)
Baetoidea	Lack of polar caps other than types IV and V	Tagenoform
Leptophlebioidea	Polar caps absent*	Funnelform
Ephemeroidea	Polar caps other than type II absent	Funnelform
Ephemerelloidea	Type I polar cap	Tagenoform
Caenoidea	Type III polar cap or absent	Linear or poorly developed tagenoform
Prosopistomatoidea	Type III polar cap, undersized, unorganized	Tagenoform

*Type I, Non-coiled, single unit cap; type II, non-coiled, multi-unit cap; type III, multi-threaded coiled cap; type IV, single-threaded coiled cap; type V, KCT cap (knob-terminated coiled threads).

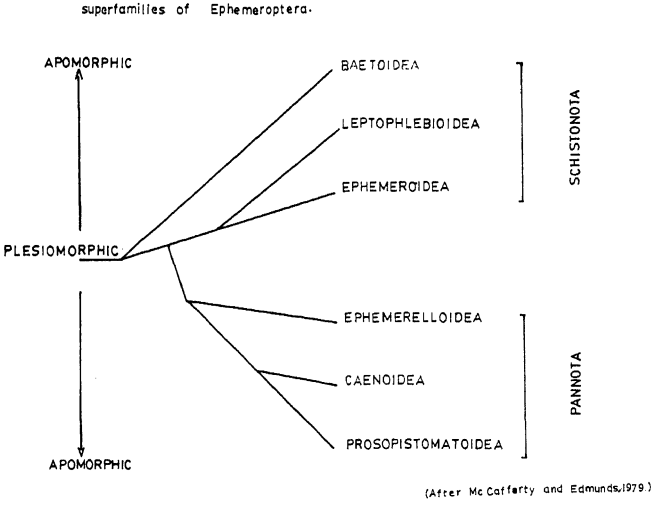

Figure 1. Phyletic diagram representing the probable phylogeny of the superfamilies of Ephemeroptera (after Mc Cafferty and Edmunds 1979).

Ephemeroptera, chorionic sculpturing evolved independently in nearly every family and superfamily; therefore, it has little use as a tool for reconstructing a phylogeny indicating familial relationships. The micropyles and attachment structures supply the bulk of the data useful for phylogenetic studies of the order.

However, the sculptural modifications of chorion of the eggs have allowed significant contributions to the taxonomy of Heptageniidae at the generic level and of Leptophlebiidae at the generic and species level. Working on the Ecdyonurid complex of the Nearctic *Heptagenia*, Flowers (1980b) has established two new genera viz *Leucrocuta* (*maculipennis* group) and *Nixe* (*lucidipennis—simplicioides* group). Among several other characters, the chorionic sculpturing of eggs have played a vital role in the separation of these two genera and in the establishment of two subgenera

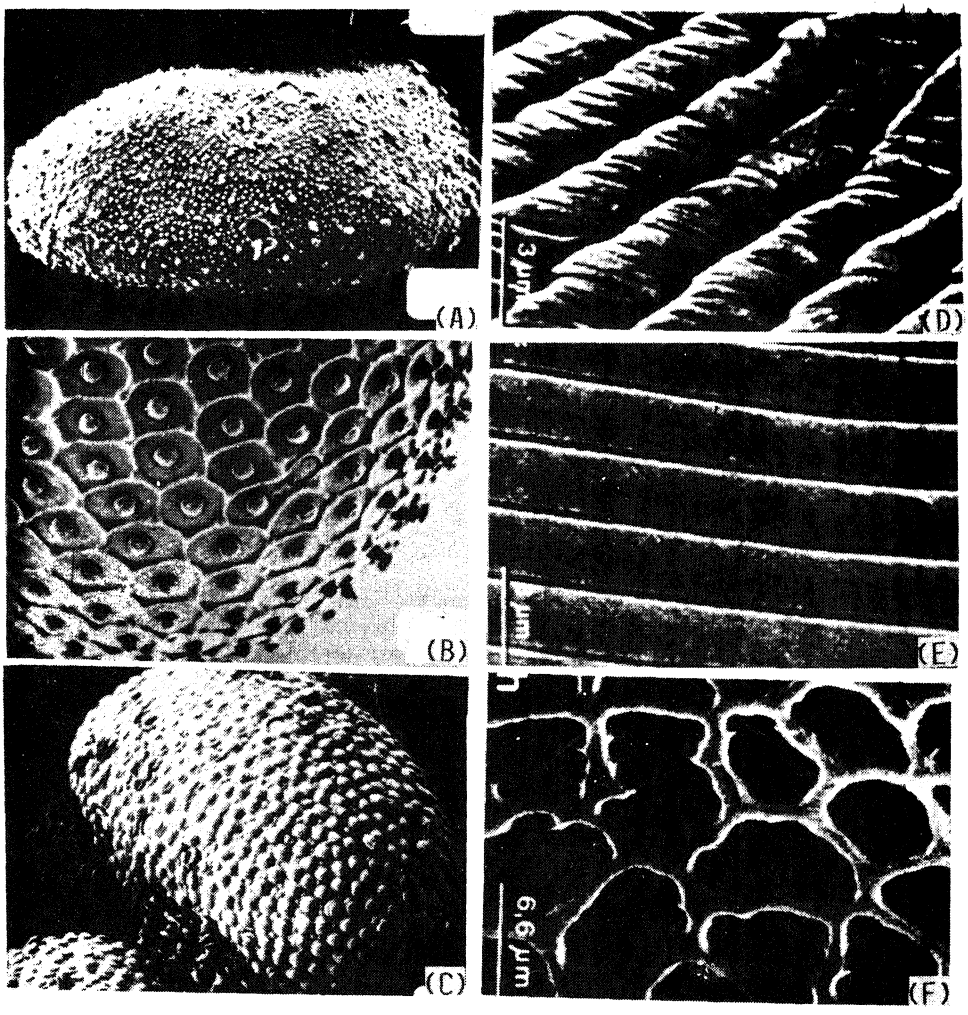

Figure 2. SEM of eggs (**A–C**, after Flowers 1980b; **D–F**, after Gaino and Mazzini 1984). **A.** *Leucrocuta juno*. **B.** *Nixe* (*Nixe*) sp. **C.** *Nixe* (*Akkarion*) *criddlei*. **D.** *Habroleptoides modesta* (chorionic details). **E.** *Habroleptoides umbratilis* (chorionic details). **F.** *Habroleptoides auberti* (chorionic details).

viz *Nixe* (*Nixe*), which belongs to the former *lucidipennis* group and *Nixe* (*Akkarion*) which belongs to the former *simplicioides* group. The chorion of *Leucrocuta juno* shows fine granular structures (figure 2A), that of *Nixe* (*Nixe*) sp. has large mesh reticulation (figure 2B) and that of *Nixe* (*Akkarion*) *criddlei* has small knobs (figure 2C) and there is no reticulated mesh. Gaino and Mazzini (1984) have shown the importance of chorionic sculpturing of the eggs in the separation of 3 species of *Habroleptoides*. *H. modesta* has slightly raised costae, about 1·8 μm wide, slightly twisted along its longitudinal axis appearing as a thin banding with very small interspaces between adjacent costae (figure 2D). *H. umbratilis* egg shows slightly raised costae arranged very close to each other on the chorion. Each costa is 1·6 μm wide, and unlike the previous species, shows no banding (figure 2E). On the other hand, the chorionic surface of the egg of *H. auberti* shows a reticulation made up of irregular polygons the sides of which vary in length but are about 1μm wide and thick (figure 2F).

3. Materials and methods

Eggs from the final instar nymphs of investigated species (from an undescribed female imago in the case of *Nathanella indica*, collected from Palni hills) were dis-

Table 2. Ultrastructural features of the eggs of some south Indian Leptophlebiidae and Heptageniidae.

	Chorion	Attachment structures	Micropyle
Family Leptophlebiidae			
Petersula courtallensis	Punctate with floristic pattern of ridges	Absent	Funnelform
Choroterpes (*Euthraulus*) sp	Ornamented with a pattern of star-shaped bosses, each with 8–9 irregular raised ribs radiating from the centre	Absent	Funnelform
Thraulus gopalani	Thick without ornamentation	Each pole of the oval egg with 8 very long, stout curved spinous processes	"
Indialis badia	Ornamented with longitudinal undulating ridges	Circular, sucker-like at both poles	"
Genus nov. sp. nov.	Tuberculate	Peglike structures	"
Nathanella indica	Reticulate	Absent	Not observed
Notophlebia jobi	Tuberculate	Uniformly clothed with cylindrical, slender rods	Funnelform
Isca (*Isca*) sp.	Circular, floristic patterns	Absent	Not observed
Family Heptageniidae			
Epeorus sp.	Smooth	Absent	Tagenoform
Cinygmina sp.	Tuberculate	Large KCT equatorial	"
Thalerosphyrus sp.	Tuberculate	KCT concentrated at poles	"

sected out into 95% ethyl alcohol and transferred to 50/50 ethyl alcohol—amyl acetate and then on to 100% amyl acetate. Eggs were dried with CO_2. SEM studies were carried out in the School of Biological Sciences, Madurai Kamaraj University, Madurai.

4. Results and discussion

The ultrastructural features of the eggs of investigated species of south Indian Leptophlebiidae and Heptageniidae are given in table 2.

The only characters typical of the 3 heptageniid genera studied are a tagenoform, usually entirely chorionic micropyle and a lack of polar caps other than type V. The type of attachment structure along with the chorionic ultrastructure give some clue to distinguish the 3 investigated genera (figure 3A–C) at least in the regional peninsular Indian context.

The eggs of the investigated species of south Indian Leptophlebiidae have the greatest diversity in chorionic sculpturing (figure 4A–H). The chorion may be smooth (figure 4C), tuberculate (figure 4E), punctate with floristic pattern of ridges (figure 4A), reticulate (figure 4F) or even sculptured with a floristic pattern of ridges

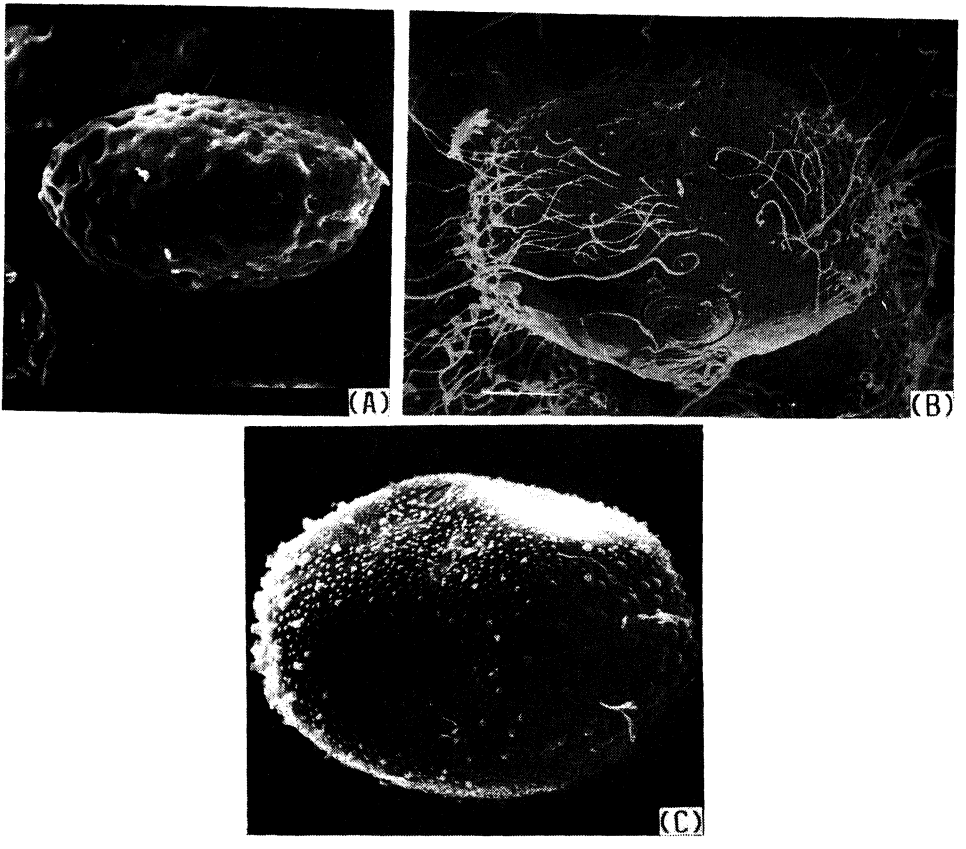

Figure 3. SEM of eggs. **A.** *Epeorus* sp. **B.** *Cinygmina* sp. **C.** *Thalerosphyrus* sp.

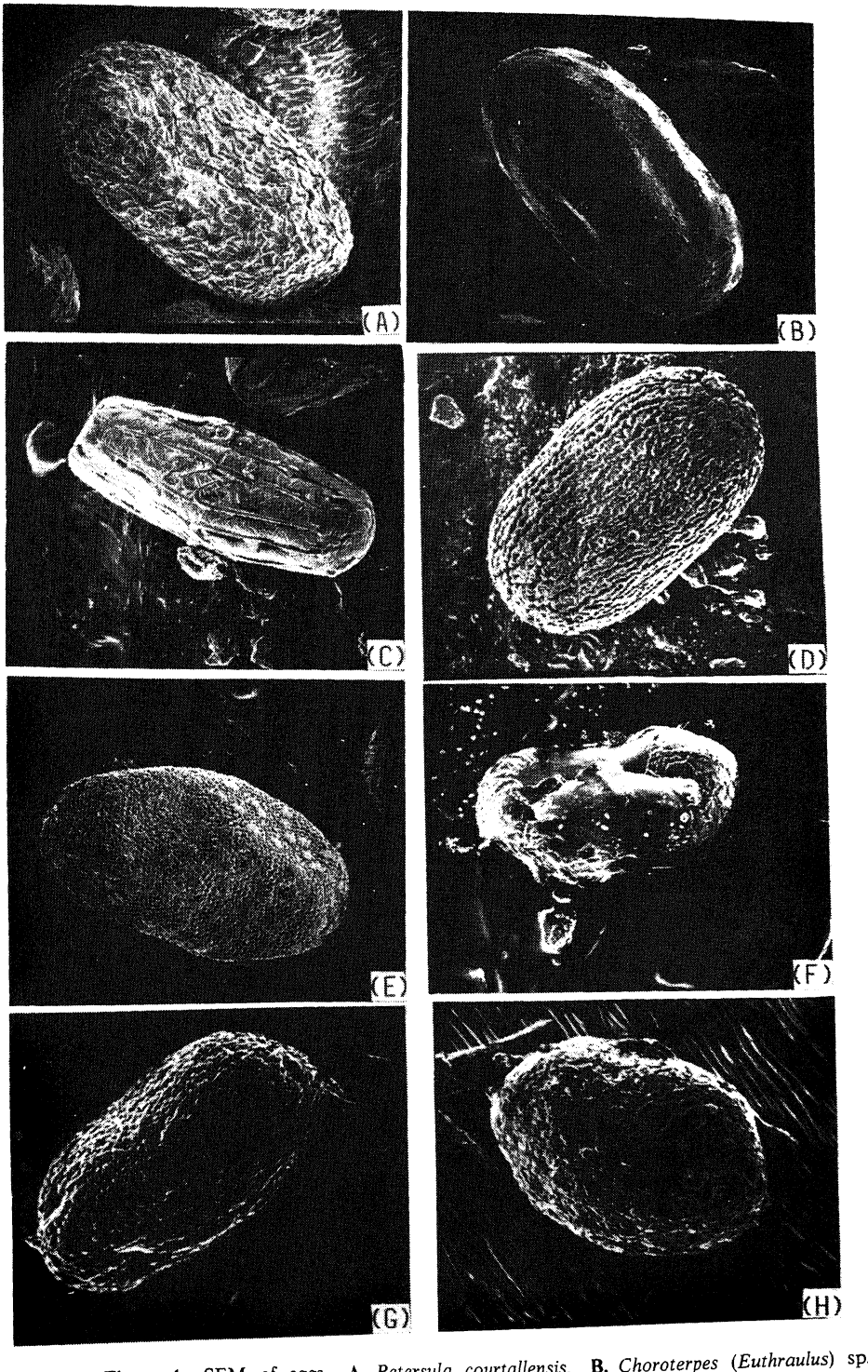

Figure 4. SEM of eggs. **A.** *Petersula courtallensis.* **B.** *Choroterpes* (*Euthraulus*) sp. **C.** *Thraulus gopalani.* **D.** *Indialis badia.* **E.** Genus nov. sp. nov. **F.** *Nathanella indica.* **G.** *Notophlebia jobi.* **H.** *Isca* (*Isca*) sp.

(figure 4H) or ornamented with longitudinal undulating ridges (figure 4D). Attachment structures are rather rare. Peglike structures (figure 4E), long, stout, curved, spinous processes around each pole (figure 4C), circular, sucker-like structures at both poles (figure 4D) and cylindrical slender rods (figure 4G) have been observed. Polar caps apparently do not occur. A funnelform micropyle (figure 4D) and lack of polar caps are the only features that are constant on all the observed species of Leptophlebiidae eggs. The chorionic sculpturing of the eggs is very useful in differentiating 8 genera of Leptophlebiidae under investigation and the data from the egg ultrastructure are consistent with the phylogenetic scheme proposed by Peters and Edmunds (1970) for eastern hemisphere Leptophlebiidae (figure 5).

Total egg data in the present study appears to be consistent with the classification and with the phylogenetic scheme (figure 1) proposed by Edmunds and his co-workers (Edmunds and Traver 1954; Edmunds 1962, 1972; Edmunds *et al* 1963; Mc Cafferty and Edmunds 1979) for the superfamilies of Ephemeroptera. The phylogeny suggested by the egg data is inconsistent with the classification proposed by Demoulin (1958). The present study confirms the earlier observation of Koss and Edmunds (1974) that the Leptophlebiidae with their funnelform micropyle are most certainly of enough distant relationship with the Heptageniidae which have tagenoform micropyle to warrent inclusion in separate superfamilies viz Leptophlebioidea and Baetoidea.

The subfamily Atalophlebiinae of the family Leptophlebiidae has undergone extensive adaptive radiation in peninsular India. Many genera like *Choroterpes*, *Thraulus*, *Indialis*, *Notophlebia* and *Isca* apparently have several species complexes and judicious use of data from egg ultrastructure along with other modern biosystematic tools is bound to help in solving many taxonomic and phylogenetic puzzles in future.

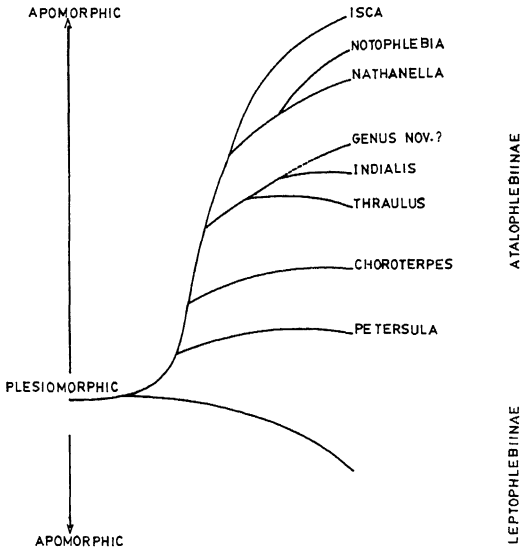

Figure 5. Phyletic diagram representing the probable phylogeny of the genera of south Indian Leptophlebiidae (modified from Peters and Edmunds 1970).

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Experimental studies on the hatching rhythm and larval release in Palaemonid and Atyid prawns

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Abstract. The hatching behaviour and larval release of *Macrobrachium lanchesteri* (de Man) (Palaemonidae) and *Caridina weberi* (de Man) (Atyidae) are described in relation to natural (LD nat) and altered laboratory (LL, DD and DL) illumination cycles. A clear nocturnal hatching rhythm is observed in female *Macrobrachium lanchesteri*, which releases the larvae in batches on successive nights till all the eggs held in the brood are hatched. Maximum number of larvae are released between 24 and 3 h. In *Caridina weberi* such a precise rhythm is loosely operative.

Keywords. Light/darkness cycles; hatching rhythm; freshwater prawns.

1. Introduction

Daily fluctuations in the intensity and duration of light are known to induce adaptive changes in the hatching behaviour of decapod crustaceans (Ennis 1973, 1975; Katre *et al* 1980). However, once these entraining influences are removed and under constant laboratory conditions, a circadian rhythm is known to persist (Brown 1961). Such persistent rhythms suggest the influence of endogenous oscillators in synchronizing the behaviour with environmental cycles. Persistent endogenous hatching mechanisms have so far been reported for *Homarus gammarus* (Ennis 1973) and *Macrobrachium lamarrei* (Katre *et al* 1980). In both these cases, the nocturnal hatching mechanism is known to be only slightly altered by illumination. Presently the larval hatching behaviour of the Palaemonid, *Macrobrachium lanchesteri* and the Atyid, *Caridina weberi*, prawns have been described in relation to altered light-dark cycles. These two species of prawns are known to coexist in local freshwater habitats though occupying different ecological niches (Anantha Raman *et al* 1986). Hence, a comparative analysis of the larval hatching in the two species has been attempted.

2. Materials and methods

Ovigerous females of *M. lanchesteri* and *C. weberi* were collected from local freshwater habitats and acclimated to laboratory conditions. From the stocks prawns with an average length of $40{\cdot}8 \pm 3{\cdot}5$ mm for *M. lanchesteri* and $23{\cdot}3 \pm 1{\cdot}7$ mm for *C. weberi* were selected for studying the hatching behaviour. Two sets of experiments were conducted. In the first set, the overall hatching behaviour of the prawns was studied in relation to LD (nat) cycles. In the second set, effects of altered laboratory light-dark cycles on this hatching behaviour was studied.

2.1 *Normal hatching behaviour of prawns*

Two series of experiments were conducted. Prawns were maintained in groups of 12 (*M. lanchesteri*) and 24 (*C. weberi*) per aquarium, both in the laboratory and in the field. The different densities at which the two species of prawns were reared is related to their size difference, the former species being nearly twice as large as the latter. For each series, 12 replicates were maintained and the water in the aquaria was continuously aerated. The prawns were fed *ad libitum* on *Tubifex* worms. While the prawns maintained in the field aquaria experienced natural LD cycles, those maintained in the laboratory aquaria were exposed to artificial light-dark cycles of 12 h (between 6 and 18 h) L and 12 D. Light from a tungsten filament lamp, bounced from a reflecting dome, on to the water surface, provided the average illumination of 25 Ft.C., during the light phase of the cycle. The average temperature of water in the field/laboratory maintained aquaria was 26° and 24°C respectively. A variation in water temperature of 2°C is a natural phenomenon in tropical freshwaters and hence the temperature variations were not considered as important.

2.2 *Hatching behaviour in relation to altered light-dark cycles*

Females with brood of eggs nearly ready to hatch, were selected from either species and maintained in groups (4 and 8/15 l freshwater for *M. lanchesteri* and *C. weberi* respectively). These were exposed to different light-dark cycles: LD nat (12 L: 12 D), DL (reversal of LD), LL (constant light) and DD (constant darkness). The light-dark phases were provided by using an auto-reset synchronous timer (Model: GIC-EM 1000) in an experimental chamber (size: 180 × 60 × 65 cm) specially designed for the purpose. During the dark phase, a low wattage red light source was used to locate the larvae. The number of larvae released by each group of prawns were visually recorded once every 3 h during the successive 24 h cycles. At each time interval, the larvae released were collected through specially designed pipettes and the number of larvae released were expressed as number/female/h. The observations were continued till the females hatched all the eggs held in the brood and underwent the post-parturial moult.

3. Results and discussion

3.1 *Normal hatching rhythm of prawns*

Table 1 presents the data on brood size and larval release in the two species of prawns. The brood size of *M. lanchesteri* is significantly less than that of *C. weberi*. The Atyid prawns (*C. weberi* and *C. nilotica*) are known to be more fecund than Palaemonid prawns (*M. lamarrei*: see Ponnuchamy *et al* 1979; Rao *et al* 1981). From table 1 it is also interesting to note that, despite the larger size and lower fecundity, *M. lanchesteri* extended the duration of larval release to 3 days, while in *C. weberi*, it was limited to only two days. Further, irrespective of the experimental conditions, *M. lanchesteri* released the bulk of its larvae on day 1 and the percentage egg hatching decreased significantly on days 2 and 3. As compared to this, *C. weberi* exhibited no definite pattern of larval release in relation to the day.

Table 1. Brood size and number of batches of larvae released by *M. lanchesteri* and *C. weberi*.

Species	Experimental series	Length range of prawns (mm)	Brood size range (No. of eggs)	Number of larvae released during day		
				1	2	3
M. lanchesteri	A	36–42	39–68	26–51 (71·15)	4–33 (18·59)	2–19 (10·26)
	B	40–46	36–102	19–79 (70·38)	1–30 (23·91)	2–12 (5·71)
C. weberi	A	21–26	313–625	33–409 (30·95)	43–543 (61·05)	—
	B	21–24	303–395	106–395 (62·49)	82–271 (37·51)	—

Observations were made on individually maintained prawns are 6 h on successive mornings. The numbers in parentheses indicate the average percentage larval release in relation to the brood size.
A, Laboratory maintained prawns; B, field maintained prawns.

The larval hatching behaviour of the two species of prawns maintained in groups in the laboratory/field are presented in figure 1. In either experimental conditions (figures 1 A_1 and A_2) *M. lanchesteri* released more larvae during the dark phase than during the light phase, thus exhibiting a well timed noctural rhythm in its hatching behaviour. However, *C. weberi* did not exhibit this pattern and released almost same percentage of larvae both during the light and dark phases of the LD (nat).

The pattern of egg hatching and larval release of individually maintained *M. lanchesteri* and *C. weberi* (figures 1 B_1 and B_2) was similar to those maintained in groups further implicating that the observed behaviour has no bearing on the density of prawns maintained in the experimental series.

3.2 *Larval hatching in different illumination cycles*

The larval release behaviour of females of *M. lanchesteri* in relation to LD (nat), LL, DL and DD cycles and maintained in groups are presented in figure 2. In LD (nat; figure 2A), nocturnal hatching appears to be characteristic of all ovigerous females of *M. lanchesteri*. Perhaps onset of darkness acts as a 'zeitgeber' in synchronizing the nocturnal rhythm of hatching. A comparable nocturnal pattern has also been indicated for *M. lamarrei* (Katre *et al* 1980) and *Homarus gammarus* (Ennis 1973). Peak larval release by *M. lanchesteri* occurred between 24 and 6 h. When maintained in DL cycles, from about 6 h prior to the first larval release, *M. lanchesteri* initially released some larvae during the day phase still corresponding to the light phase of the LD (nat; figure 2A). Subsequently, the prawns completely synchronized the pattern to coincide with the dark phase (corresponding to the night phase of the LD (nat) indicating a complete reversal in the DL cycle). However, prawns maintained in the LL cycles (figure 2C), irrespective of the presence of light, continued to release larvae during a phase corresponding to the dark phase of the LD cycle indicating that an endogenous component may probably be responsible for regulating the rhythm. Interestingly, when the prawns were maintained in DD cycles (figure 2D),

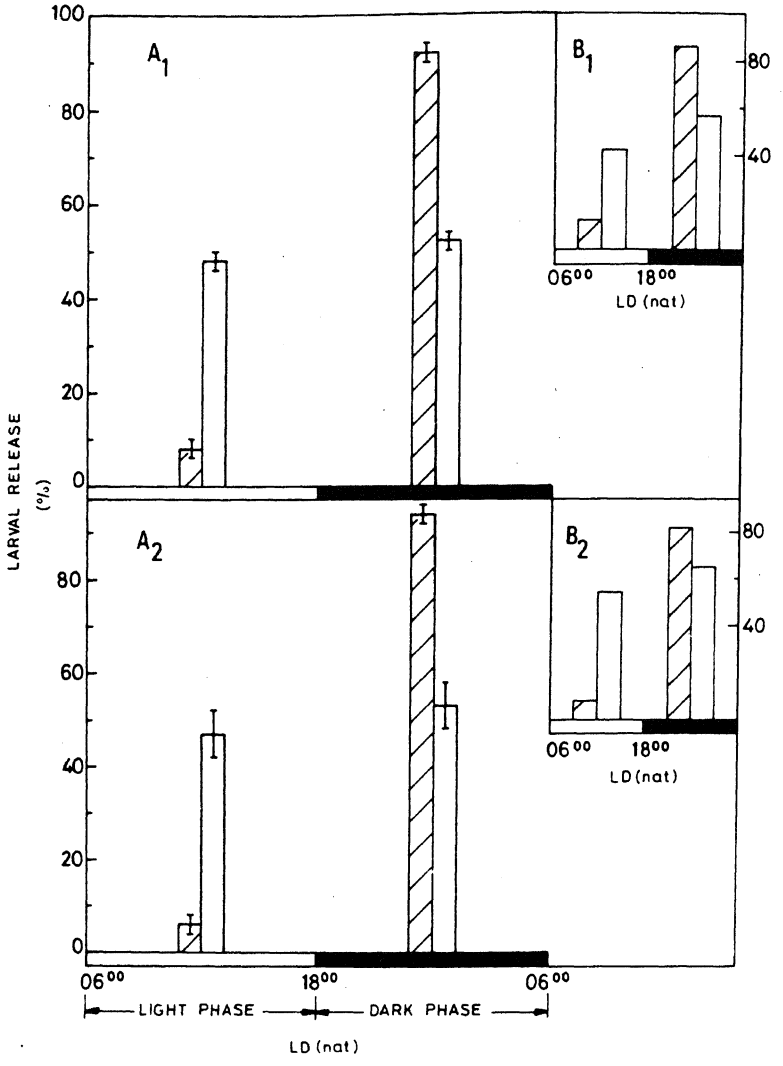

Figure 1. Hatching behaviour of *M. lanchesteri* (▤) and *C. weberi* (□) maintained in groups, in the field (A_1) and laboratory (A_2) conditions. Figures B_1 and B_2 represent the hatching behaviour of the two species of prawns maintained individually in the field and laboratory conditions respectively.

the pattern was slightly arhythmic and release of larvae occurred throughout the period. However, maximum per cent of larvae were released during a period corresponding to the dark phase of the LD (nat). Thus, in *M. lanchesteri*, onset of darkness appears to synchronize the hatching rhythm but an endogenous component may be involved in controlling the process of synchronization. This is again comparable to the findings in *M. lamarrei*. Several physiological processes of insects, fishes, reptiles, birds and mammals are known to be endogenously regulated (Kirkpatrick and Leopold 1952; Kirkpatrick 1959; Beck 1963; Halberg 1969; Winfree 1970). Probably an interaction of both exogenous and endogenous factors may control the nocturnal

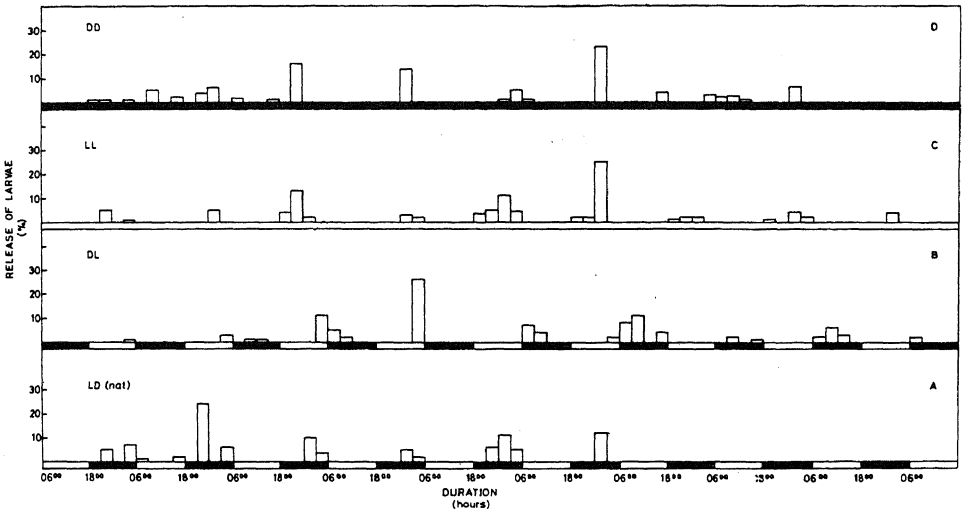

Figure 2. Hatching behaviour of *M. lanchesteri* (in groups) under natural and altered illumination cycles.

hatching behaviour and the 'gating phenomena' in the larval release behaviour of *M. lanchesteri* (see also De Coursey 1961; Pittendrigh 1961; Bunning 1967; Brown *et al* 1970).

Observations on the hatching behaviour and larval release by *C. weberi* maintained in groups and exposed to LD (nat) indicated that though larval release mostly occurred during the night phase, a significant percentage of larvae were also released, on and off, during the day phase (figure 3). This suggests that the nocturnal rhythmic larval release in *C. weberi* is not as rigid as in *M. lanchesteri*. When exposed to DL, LL or DD cycles (figure 3B–D), release of larvae by *C. weberi* occurred both during the light as well as dark phases of the cycles, further confirming a loosely operating nocturnal rhythm of larval release in this species. A loosely controlled hatching rhythm has also been observed in *Homarus americanus* (Ennis 1975).

The overall hatching behaviour of *M. lanchesteri* and *C. weberi*, in relation to different light-dark cycles confirms that the behaviour of larval release in *M. lanchesteri* was unaltered by the constant light phase (LL) and that dark phase (DL and DD) played an important role in synchronizing the pattern of hatching. This confirms that the nocturnal hatching rhythm is triggered partly by the exogenous factor 'darkness' through the mediation of an endogenous component(s) (probably neurohormonal). Comparatively, *C. weberi* shows only a less specific nocturnal hatching rhythm, perhaps suggesting that the endogenous component may not be as precise in controlling the process as in *M. lanchesteri*.

It is interesting to know how embryonic development in *M. lanchesteri* is so accurately synchronized to release larvae night after night. A precise control is lacking in *C. weberi* which releases larvae throughout the night and also at different times during the day. The significance of this difference in these two natantians, co-existing in freshwater habitats is interesting. Adults of *M. lanchesteri*, especially the ovigerous females are basically bottom dwellers (Johnson 1966). Obviously, the animals tend to synchronize the larval release to the dark phase when exposed to the

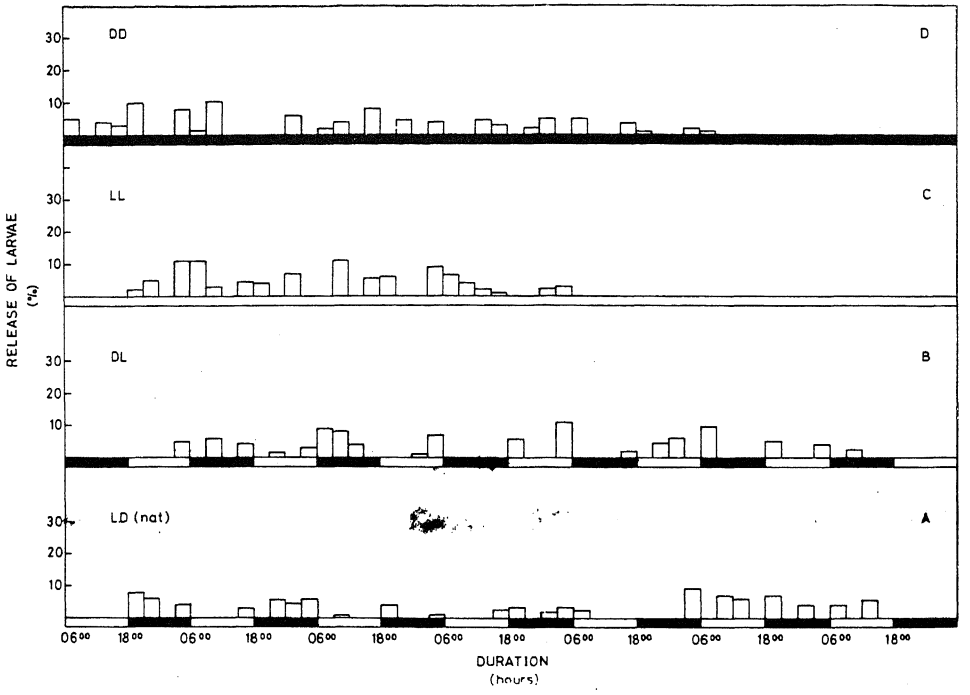

Figure 3. Hatching behaviour of *C. weberi* (in groups) under natural and altered illumination cycles.

LD cycles. However, the Atyid *C. weberi* is generally found in marginal/surface zones along with submerged vegetation (Anantha Raman *et al* 1986) and normally, light may not have a significant bearing on the timing of physiological activities of the species. Hence, even larval release is not strictly synchronized with any particular (light or dark) phase of the LD cycles. In any case, for either species, release of larvae during the night phase may have definite advantages of a better avoidance from predators and a better survival rate (see also Bergin 1981). So, there is a natural tendency in both the species to release more larvae during the night phase of the LD cycles, but the niche characteristics render the noctural rhythm more precise in *M. lanchesteri* than in *C. weberi*. In *M. lanchesteri*, mating and spawning is restricted to a single night (Rao 1983) and it is interesting how the observed behaviour of staggered larval release can account for the finely regulated embryonic development in the species.

3.3 *Egg fanning*

Visual observations and counts were made on the rate of 'pleopod beating' (egg fanning) in the two natantians in relation to the incubation stage of the eggs. Ovigerous females of *M. lanchesteri* and *C. weberi* were found to raise themselves slightly on their walking legs and with the abdomen fully extended horizontally, beat the pleopods gently. This mode of 'pleopod beating' or 'egg fanning' is a specific behaviour that appears to be an integral part of the normal incubation/hatching

process of *Macrobrachium* spp. (Katre and Pandian 1972; Katre 1973; Katre *et al* 1980), *Palaemon serratus* (Phillips 1971) and *Homarus gammarus* (Ennis 1973) and is important in irrigating the eggs. The pleopod beating was exhibited by the adult female, only during the brooding period, beginning with spawning and brood pouch formation and ending with the post-parturial moult (Katre *et al* 1980). The rate of pleopod beating was found to vary with the developmental stages of eggs in the brood pouch, increasing with advance in development (*M. lanchesteri*: figure 4; *C. weberi*: figure 5). Further, in the two species the rate of pleopod beating by the females carrying eggs in the final stages of the development was higher during the dark phase than in light phase. A similar behaviour of pleopod beating in relation to larval release has also been reported for *M. lamarrei* (Katre *et al* 1980). While this suggests a probable role for the ovigerous females of these species of prawns in synchronizing the rigid or loosely operating nocturnal hatching rhythm, egg fanning alone may not be the controlling factor in the embryonic development.

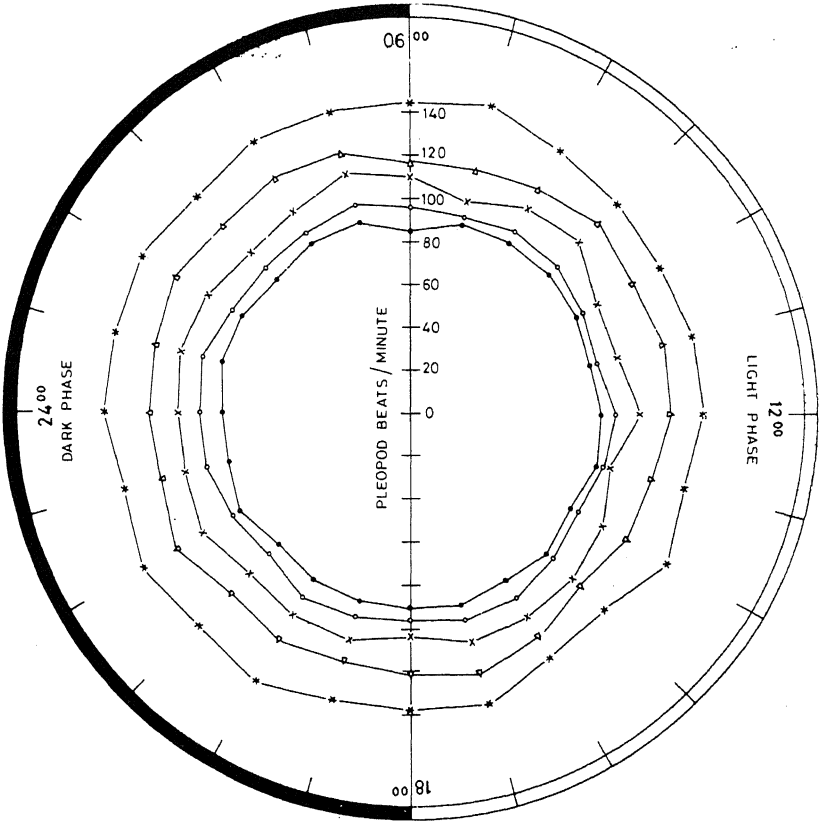

Figure 4. Rate of pleopod beating during the brooding period of an ovigerous *M. lanchesteri* in relation to the light-dark phase of the natural illumination cycle and the stage of developing eggs.

I stage (●): Eggs soon after spawning, contain oil globule and deep green in colour.
II stage (○): Eggs indicating differentiation of blastoderm at one end.
III stage (×): Pigmented eyes seen in the developing eggs.
IV stage (△): Clear pigmented eyes, egg translucent.
V stage (★): Occasional movements of the embryo in the egg.

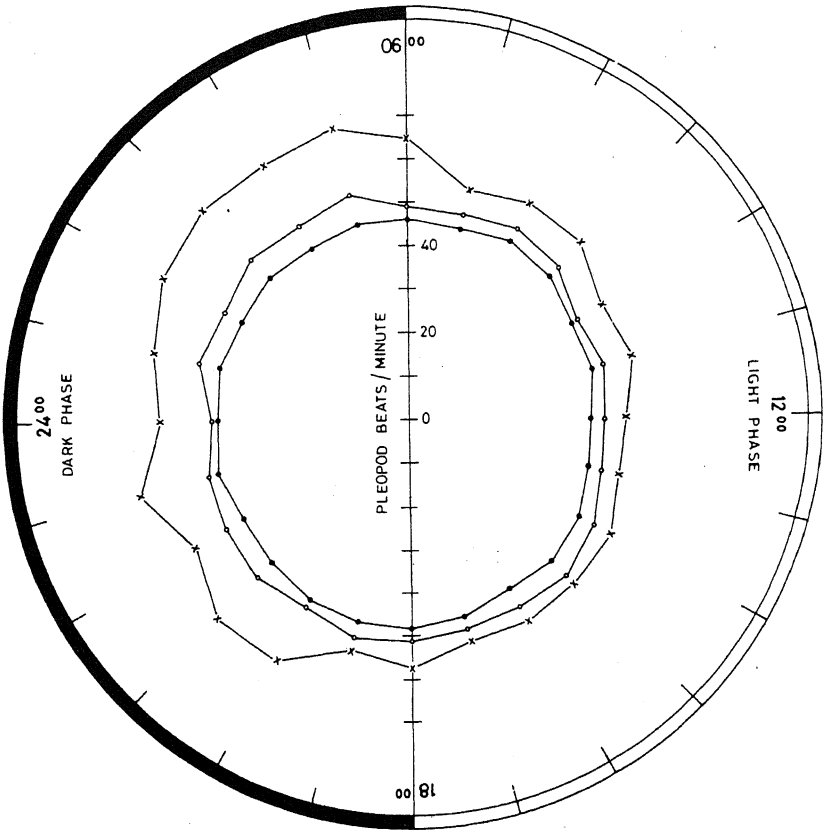

Figure 5. Rate of pleopod beating during the brooding period of ovigerous *C. weberi*, in relation to the light-dark phase of the natural illumination cycle and the stage of developing eggs.

I stage (●): Eggs soon after spawning, contain oil globule and vary from red to brown in colour.

II stage (○): Eggs indicating differentiation of the blastoderm at one end, slightly translucent.

III stage (×): Eggs fully translucent, pigmented eyes well formed, occasional movements of the embryo.

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Population structure and breeding season in *Rattus rattus wroughtoni* Hinton*

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Abstract. Observations were made from July 1982 to June 1985 on certain aspects of the population ecology of *Rattus rattus wroughtoni* Hinton. Altogether 2639 specimens of rats were collected. The male to female ratio was 0·87 : 1·0. Male rats were significantly heavier and bigger than the females. Pregnant female rats were encountered throughout the year with two peaks, one during early summer (February–March) and the other during monsoon (July–August). The incidence of pregnancy was lowest in May when the day temperature was maximum. The litter size ranged from 1–8. The pre-implantation loss was 0·75 ovum per female.

Keywords. *Rattus rattus wroughtoni*; sex ratio; body weight; breeding season; litter size; pre-natal mortality.

1. Introduction

Rattus rattus wroughtoni Hinton is the most common murid distributed in the south west India (Ellerman 1961). It is one of the major rodent pests of plantation crops, especially coconut (Advani 1985) and cacao (Bhat 1978; Abraham and Remamony 1979; Bhat *et al* 1981). Observations on its nests and breeding behaviour in captivity (Rajagopalan 1970, 1972) and some aspects on its population and post-natal development (Advani 1984b) were already made. In this communication, we discuss the data collected for 3 years on the population structure and breeding season of *R. r. wroughtoni.*

2. Study area and climate

The study was conducted in the plantations of coconut, arecanut and cacao around Kasaragod (12° 30′ N; 75° E). Other crops such as banana, pineapple, black pepper, jack and mango were also intercultivated. During the rainy season, the undergrowth in the plantations was quite thick with annual weeds, but during summer most of these dried up and the soil surface was relatively devoid of any ground cover. The climatic conditions of this area are given in figure 3.

3. Methods

Monthly collections of live specimens of *R. r. wroughtoni* were made at random, from different plantations during July 1982 to June 1985 using wooden box traps. The trapped animals were removed daily, brought to the laboratory and data collected

*Contribution No. 577.

on their sex, body weight and linear measurements of head and body, tail, pinna and hindfoot. The animals were then dissected and the following aspects were studied and record maintained individually. In males the position of the testes (abdominal or scrotal) was noted and smears from the cauda epididymus were examined for the presence of sperms. In females, the embryos and corpora lutea, if present, were counted. The male rat was considered to be reproductively active if the testes were scrotal and sperms present in the smears of its epididymus and the female considered mature if its vagina was perforate (Tamarin 1977). Ovulation rate and litter size were determined from the number of corpora lutea in the ovaries, and implanted embryos in the uteri respectively. Pre-implantation loss was calculated as the difference between the total number of corpora lutea and the embryos in the animals displaying both. Post-implantation loss due to the mortality of implanted embryos was determined by counting the resorbing embryos.

4. Results and discussion

4.1 *Sex ratio*

Out of 2639 specimens of *R. r. wroughtoni* collected 1231 were males and 1408 were females (0·87 : 1·0). This showed a significantly more ($X^2(1) = 11{\cdot}6$; $P < 0{\cdot}001$) number of females in the population. A similar trend was reported in several other populations of *R. rattus* (Iyer 1933; Deoras *et al* 1975; Advani 1984a; Advani and Rana 1984). In *R. rattus* (Ecke 1955; Bently and Taylor 1965), *R. norvegicus* (Davis 1948) and in *R. meltada* (Chandrahas and Krishnaswami 1974) the females were known to persist longer (nearly 1·3 times) than the males in the population. Such difference in longevity in favour of females could be the main reason for the presence of more number of females in the population.

The males outnumbered the females only during March, July and August (figure 1). This could be attributed to the presence of more number of pregnant females during these months as compared to the whole year (figure 2). Barnett and Prakash (1975) established that the movements of female rats were much restricted few days before and after parturition. This restricted movements in females might have reduced the trapping success in females.

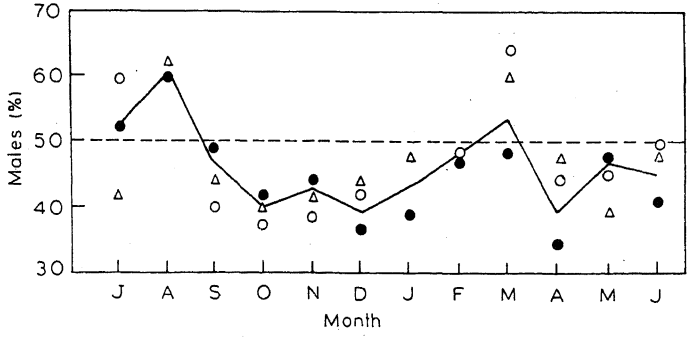

Figure 1. Sex ratio (% of males) of *R. r. wroughtoni* in different months. (●), 1982–83; (○). 1983–84; (△), 1984–85. The line represents the pooled data for 3 years.

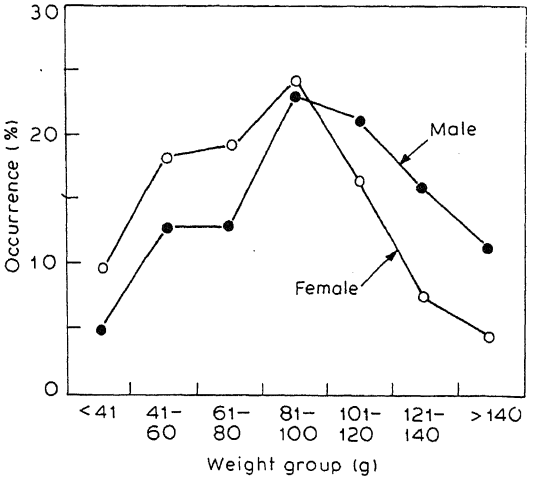

Figure 2. Percentage of occurrence of various weight groups in *R. r. wroughtoni*.

Table 1. Sex ratio of *R. r. wroughtoni* in different weight groups.

Weight group (g)	Sample size	Number		Males (%)	$X^2(1)$	*P* value
		Males	Females			
<41	189	61	128	32·3	23·7	0·001
41–60	420	158	262	37·6	25·7	0·001
61–80	428	160	268	37·4	27·2	0·001
81–100	625	283	342	45·3	5·6	0·05
101–120	495	263	232	53·1	1·9	NS
121–140	273	166	107	60·8	13·6	0·001
>140	209	140	69	67·0	24·1	0·001

When the animals were grouped into separate weight classes (table 1) it was revealed that the female rats were significantly more in number than the males in lower weight classes (< 100 g), whereas the males significantly outnumbered the females in higher weight groups (> 121 g). Bently and Taylor (1965) have reported that in *R. rattus* the males gained weight much faster than the females during their development. Thus, the faster weight gain in males might have resulted in the presence of significantly more number of heavier males than females in the population. Chandrahas and Krishnaswami (1974) and Okia (1976) were also of the opinion that in rats the males acquire body weight faster than the females.

4.2 *Body weights*

In general, the male rats were significantly heavier and bigger ($P < 0{\cdot}01$) than the female rats (table 2). The body weight in male rats varied from 20–190 g and in female rats from 20–176 g. Disparity in mean body weights in favour of male rats was also reported in other *Rattus* group (Bently and Taylor 1965; Chandrahas and

Table 2. Body weight and morphological measurements of *R. r. wroughtoni.*

Sex	Sample size	Body weight (g)	Morphological measurements (mm) Head and body	Tail	Hind foot	Pinna
Male	1231	98·93 ± 1·01	140·69 ± 0·68	192·68 ± 0·91	31·45 ± 0·10	19·76 ± 0·08
Female	1408	85·43 ± 0·91	133·04 ± 0·85	183·49 ± 0·92	30·82 ± 0·09	19·36 ± 0·07
CD at $P = 0·05$		2·68	1·86	2·57	0·27	0·21

Table 3. Percentage of pregnancy in different weight groups of female *R. r. wroughtoni.*

Weight group	Sample size	Perforat females Number	%	Pregnant females Number	%
<41	128	—	—	—	—
41–60	262	21	8·02	—	—
61–80	268	227	84·70	13	5·73
81–100	342	342	100·00	51	14·91
101–120	232	232	100·00	64	27·59
121–140	107	107	100·00	30	28·40
>140	69	69	100·00	18	26·87
Total	1408	998	70·88	176	17·67

Krishnaswami 1974). Okia (1976) attributed such disparity in body weights to child bearing which slows down the weight gain in females. Nearly 46% of male rats weighed >100 g whereas only 29% of the female rats attained this weight (figure 2).

4.3 *Male reproductive condition*

Examination of the testes and epididymal smears in the male rats revealed that they were reproductively active throughout the year. The body weights of those animals with scrotal testes and sperms in the epididymal smears ranged from 58–190 g (mean 114·9 ± 2·75 g); whereas those animals with abdominal testes and without sperms in the epididymal smears weighed from 20–65 g (mean 47·8 ± 0·85 g).

4.4 *Female reproductive condition*

The presence of female rats with perforate vaginae indicated that they were receptive all the year round. The rats with perforate vaginae weighed between 60 and 176 g (mean 100·8 ± 0·74 g) and those with imperforate vaginae weighed between 20 and 66 g (mean 46·5 ± 0·62 g). The percentage of perforate females in different weight classes is given in table 3. Out of the 998 perforate female rats 176 (17·6%) were found to be pregnant. Pregnancy was observed throughout the year (figure 3) with two peaks one during February–March (early summer) and the other during July–August (monsoon). The rate of pregnancy was lowest during summer months (April to early part of June). Similarly uninterrupted breeding was noticed in *R. r. wroughtoni*

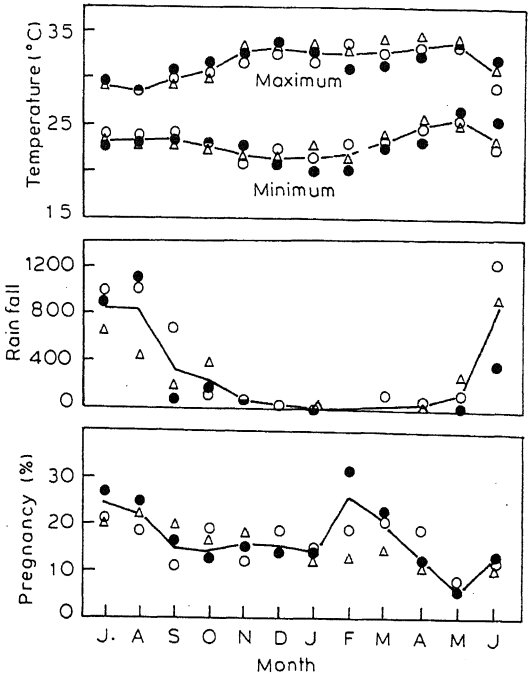

Figure 3. Percentage of total perforate female *R. r. wroughtoni* pregnant in each month. See figure 1 for caption. Data correlated with the weather conditions.

in Hyderabad (Jain 1979) and in *R. r. rufescens* in Pune (Pradhan 1982). Even in the caged condition the *R. r. wroughtoni* was reported to breed throughout the year (Rajagopalan 1972).

The observed percentage of pregnancy in *R. r. wroughtoni* ranged from 6·6 in May to 26·4 in February. In rodents it is not possible to visually detect early stages of pregnancy as the embryos become visible only after the sixth day of pregnancy as bulges in the uterine horns (Keller and Krebs 1970; Tamarin 1977). The gestation period in *R. r. wroughtoni* was 25 days (Rajagopalan 1972). Hence the percentage of pregnancy calculated by the visual observations on the presence of embryos will be about 24% less than the actual value.

In May, the day temperature was maximum (33·5°C). Consequently, the availability of food and water was also less in this month as compared to that in other months. This could be the main reason for the decline in breeding intensity. The peak in breeding was in February which also coincided with the lowest day temperature. During monsoon, though the day temperature was low the torrential rains (>2600 mm in 3 months) restricted the activity and food collection in these rats. Consequently the rate of breeding during monsoon was not as high as in February. Under similar climatic conditions at Vittal (about 65 km NE of Kasaragod) the breeding intensity in the western ghat squirrels was also very low during monsoon (Bhat and Mathew 1984).

When the pregnancies were correlated with the body weights of female rat (table 3) it revealed that nearly 72% of the breeding females weighed >100 g. The

pregnancy was witnessed even at 61–80 g weight class, but the frequency was only around 6%.

4.5 *Juvenile emergence*

The juveniles were seen in the population throughout the year (table 4). This could be attributed to the presence of pregnant females in all the months (figure 3). However, the percentage of juvenile recruitment was little more than the mean during February, April to June and October–November. The young ones of *R. r. wroughtoni* required nearly two months to attain a body weight of about 40 g (Rajagopalan 1972; Advani 1984b). Hence the breeding peak during February–March and July–August might have resulted in the emergence of more number of juveniles during April to June and October–November.

4.6 *Ovulation rate and litter size*

Altogether 176 pregnant female rats were observed for embryos and corpora lutea. The corpora lutea count varied from 1–9, whereas the embryo count varied from 1–8 (figure 4). In corpora lutea counts the majority (67%) of the animals had 5 or more ova but because of the loss of some ova before implantation more than 53% of the animals showed less than 5 embryos. The mean number in these observations was $5{\cdot}24 \pm 0{\cdot}16$ ova and $4{\cdot}49 \pm 0{\cdot}10$ embryos, respectively. Similarly in Andhra Pradesh the black rats were known to have a litter size of 1–9 (Jain 1979). Based on the number of young ones at birth Rajagopalan (1972) had reported a litter size of 1–5 in this rat.

Unilateral implantation was noticed in 11 (6·25%) instances. However, there was no preferential distribution of embryos between the horns.

4.7 *Pre-natal mortality*

This includes two aspects, pre- and post-implantation loss. Out of 176 female rats observed 106 (60·2%) showed pre-implantation losses. In 83 animals one ovum, in 17

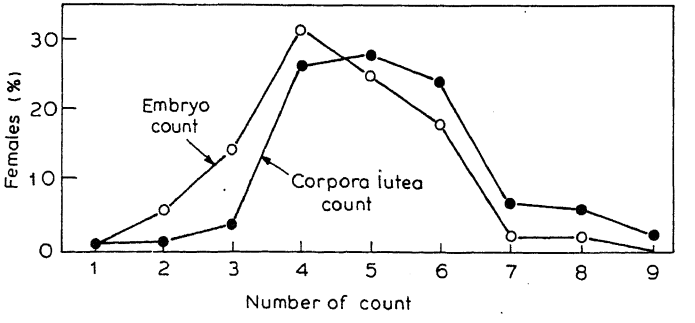

Figure 4. Percentage of females showing various numbers of corpora lutea and embryos in *R. r. wroughtoni*.

Table 4. Monthwise distribution (percentage) of various weight classes in the population of *R. r. wroughtoni* (both sexes pooled together).

Weight group (g)	Months												
	July	August	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Mean
<41	5·3	4·5	2·7	10·0	9·0	6·4	6·7	8·7	3·4	14·6	7·5	8·6	7·16
41–60	21·7	11·6	7·3	15·6	13·7	16·7	21·4	17·1	12·1	13·9	20·2	21·1	15·91
61–80	16·0	23·9	10·0	13·0	10·7	15·7	14·6	17·9	20·0	16·7	20·8	22·3	16·22
81–100	23·8	27·7	17·4	22·6	19·4	23·0	26·4	25·8	28·3	27·8	23·7	22·3	23·68
101–120	16·0	21·3	20·5	20·0	16·4	19·6	19·7	20·8	21·9	12·5	20·2	15·4	18·76
121–140	12·1	6·5	19·2	10·7	14·6	10·3	4·5	5·4	11·7	9·7	5·2	7·4	10·34
>140	5·0	4·5	22·8	8·2	16·1	8·3	6·7	4·2	2·6	4·9	2·3	2·9	7·92
Sample size	281	155	219	270	335	204	178	240	265	144	173	175	2639

animals two ova in 4 animals 3 ova and in two animals 4 ova were lost. The loss of ovum per female was calculated to be 0·75.

Post-implantation loss was noticed in 5 (2·8%) instances in the sample of pregnant rats. However, the estimate is conservative because: (i) early resorption may be overlooked in examinations of near terminal pregnancies, (ii) embryos destined to resorb in a later stage may be overlooked in examinations of early pregnancies and (iii) resorption of whole litter would be missed entirely. Thus, only pregnant females with visibly resorbing embryos were included in the post-implantation analysis.

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Feeding and extracellular digestive rhythms in some intertidal bivalve molluscs

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Abstract. The tidal rhythms for changes in morphology, length, dry weight and protein content of the crystalline style are studied in two venerid clams, *Meretrix meretrix* and *Katelysia opima* and in a donacid clam, *Donax cuneatus*. In the two venerid clams the style is housed in a sac conjoined with midgut and there is a tidal rhythm of feeding and extracellular digestion involving a partial dissolution of the style in the ebb tide and reformation in flood tide. But in the donacid clam where the style sac is completely separated from midgut, no detectable changes in the style parameters are recorded during a tidal cycle. However, the rhythmicity in feeding activity is indicated, by the presence of 'mudcap' and food particles in the style during flood tide and their subsequent absence during ebb tide. Hence the tidal rhythm of feeding and digestion in *Donax cuneatus* may be without corresponding changes in the crystalline style. Also this study throws light on the mode of style dissolution.

Keywords. Crystalline style; extracellular digestive rhythm; tidal rhythm; *Meretrix*; *Katelysia*; *Donax*.

1. Introduction

Feeding and digestive processes in bivalve molluscs are generally considered to be continuous and simultaneous provided that the environmental conditions are favourable (Owen 1966; Purchon 1968). But recent studies by Morton (1973, 1983) have suggested that many bivalves from mid-tidal to sub-littoral zone have clearly defined cycles of feeding and extracellular digestion in stomach and intracellular digestion in digestive diverticula. Morton (1956) has shown in *Lasae rubra* and Hameed (1984) in *Cranostrea madrasensis* and *Anadara rhombea* that there is a tidal rhythm of feeding and digestion and the style is completely dissolved when the tide ebbs and reformed during flood tide. Tidal rhythm in feeding and extracellular digestion involving partial dissolutions and reformation of the crystalline style was also recorded in *Cardium edule* (Morton 1970), *Pecten maximum* (Mathers 1976) and *Mytilus edulis* (Langton 1977). The purpose of the present investigation was to find out the presence of rhythms of feeding and extracellular digestion in 3 intertidal bivalve species.

2. Materials and methods

Meretrix meretrix (Linnaeus), shell length 60–61 mm and *Katelysia opima* (Gmelin), shell length 40–42 mm were collected from the mud flats of the Vellar estuary at

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Porto Novo (lat. 11°29′ N and 19°49′ E long.) and transferred to a series of wooden quadrants that were exposed at the low tide for 3 h during each tidal cycle. The bivalves were acclimated to these conditions for a week before the commencement of the experiments. The donacid clam *Donax cuneatus* (Linnaeus), shell length 39–41 mm were sampled directly from the intertidal zone of Porto Novo sea shore. Each species was sampled at 90 min intervals for a continuous period of 15 h. The sampling periods were based on the Indian Tide Table part I, 1982.

2.1 *Length of crystalline style*

The crystalline style was extracted from each species by dissection, washed in distilled water and blotted dry. The length of the style was measured to the nearest 0·1 mm. After measuring the length, the styles were preserved in 10% formaldehyde solution.

2.2 *Dry weight*

The crystalline styles of 5 individuals from each sample were dried in a hot air oven (60°C) to constant weight. The dry weight of each style was determined on a micro-balance to the nearest 0·1 mg.

2.3 *Total protein*

The protein content of the style was determined by the modified biuret method of Raymont *et al* (1964). The percentage of protein was estimated individually for 5 styles from each sample.

2.4 *Relative size of entire style and its central core*

Formaldehyde preserved styles were used for this purpose. Cross sections (2–3 mm) were made with sharp razor at the region of maximum width. The diameter of the central granular core and that of the entire style were measured micrometrically. The ratio of the granular core diameter to the total style diameter was calculated. This study was limited to *K. opima* and *D. cuneatus*.

3. Results

The rhythmic changes in the crystalline style length, dry weight and protein content and physical consistency in relation to tidal cycle in *M. meretrix* and *K. opima* are presented in figure 1. The maximum values for these style parameters recorded during high tide regime gradually decreased during low tide. Thus in *M. meretrix* (shell length 60–61 mm) the style length fluctuated between 30·9 ± 0·8 mm and 33·0 ± 1·1 mm, style dry weight between 7·2 ± 0·7 mg and 9·4 ± 0·5 mg and style protein between 9·9 ± 0·8% and 11·9 ± 0·8% (figure 1).

The pattern of rhythmicity observed in the style of *K. opima* was essentially similar to that of *M. meretrix*. During the tidal cycle, *K. opima* (shell length 40–42 mm)

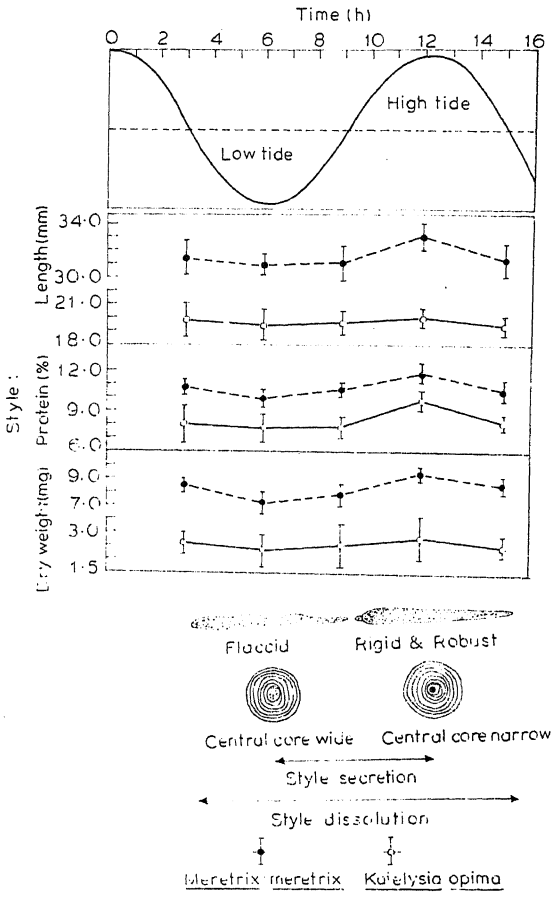

Figure 1. Rhythmic changes involving partial dissolution of the crystalline style in *M. meretrix* and *K. opima* during a tidal cycle (values are mean ± SD of 5 styles).

showed variation in the style length (19·4 ± 1·2 mm at low tide and 20·0 ± 0·7 mm at high tide), style dry weight (2·3 ± 0·6 mg in low tide and 2·8 ± 0·8 mg in high tide) and style protein content (7·7 ± 1·2% at low tide and 8·6 ± 0·8% at high tide).

The changes in the physical consistency of the crystalline style observed in *M. meretrix* and *K. opima* were also similar. During high tide the style was a firm conical transparent rod-like structure. The style head was covered with a firm 'mud cap', a mixture of organic particles and sand grains and extruded style matrix, and the body of the style embedded with food particles. The food laden mucous cord from the oesophagus was also found connected to the head of the style. During the low tide period, the style became flaccid. The 'mud cap' and adherent food particles were absent. The semi fluid central granular matrix was found oozing out from the head indicating the active process of dissolution.

Examination of cross section of *K. opima* style revealed that the style consisted of a central semi fluid granular core surrounded by a concentric rings of crystalline layers. The ration of the central core diameter to the entire style diameter during peak high tide was minimum (0·22) and maximum during low tide (0·55).

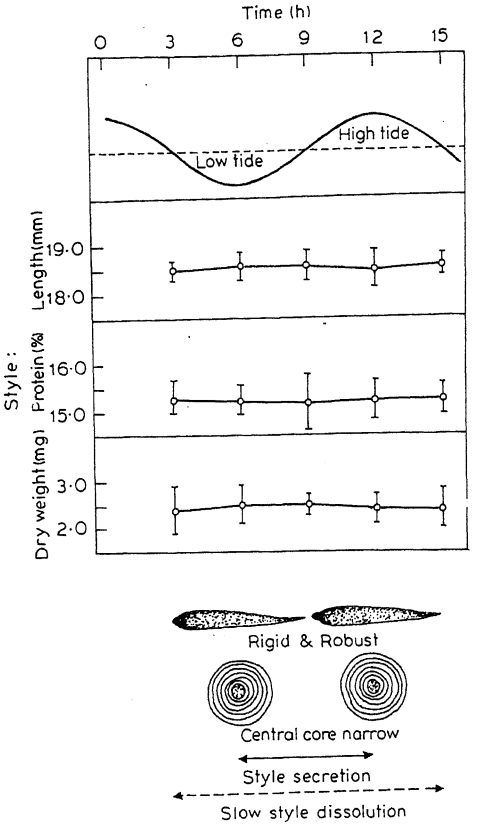

Figure 2. Changes in crystalline style of *D. cuneatus* during a tidal cycle (values are mean ± SD of 5 styles).

No detectable changes in style parameters was recorded in *D. cuneatus* during a tidal cycle (figure 2). The style was rigid and robust throughout the tidal cycle. However, the rhythmicity in feeding activity was indicated by the presence of a 'mud cap' and food particles in the style during high and their absence in low tide. The diameter of central core did not vary with the tidal cycle and the ratio of central core diameter to the style diameter remained constant (0·1) throughout the tidal cycle.

4. Discussion

The crystalline style is a prominent structure in the digestive system of most bivalve and a few gastropods. It is primarily concerned with feeding and extracellular digestion. The style is assumed to rotate and drag the food laden mucous cord into stomach and triturate it against the gastric shield (Morton 1952). The style also dissolves slowly liberating into stomach, a number of carbohydrases and lipases (Owen 1974; Morton 1983). Feeding and digestive processes in the intertidal bivalve molluscs are influenced greatly by the tidal cycle (Morton 1983). The present study indicates that the feeding and extracellular digestion are rhythmic and correlated to the phases of tide in all the 3 species tested. In *M. meretrix* and *K. opima* where the

style is housed in a style sac conjoined with midgut. The rhythmic changes in the style parameters observed in a tidal cycle suggest that style secretion is restricted to high tide regime and cessation of secretory activity may promote the style dissolution during the low tide regime. Morton (1970) established through histological study that the style of *C. edule* is secreted only during high tide. Langton and Gabott (1974) have shown in *Ostrea edulis* a correlation between the tidal cycle and changes in pH, total protein and length of crystalline style. Langton (1977) reported variation in style pH, style length and style amylase of *M. edulis* in relation to tidal cycle.

From the observation made in the present study, it is considered that the style secretion is periodic and takes place during high tide only and its dissolution is continuous throughout the tidal cycle contrary to the view of Morton (1973). Hameed (1985) reported that the optimum pH (8·0) for *in vitro* style dissolution of *M. meretrix* and *K. opima* approximately corresponds to the pH of the high tide water of the Vellar estuary and hence the style is likely to dissolve faster during high tide and releases digestive enzymes into the stomach and promote the extracellular digestion. Style dissolution during high tide has also been suggested by Mathers (1974, 1976) in *Ostrea* and *Pecten*. The visual observation of style dissolution during high tide is probably concealed since dissolution of the style is profitably compensated by the secretion of new style matrix. The increase in the central core diameter of the style of *K. opima* in low tide implies that the concentric crystalline layers become liquefied into more liquid granular central core which moves anteriorly into stomach. *In vitro* observation of the style under low power of the microscope reveals that the dissolution of style is effected by expelling the style material from the central core. This observation formed the basis for the concept of continuous style dissolution. A similar mode of dissolution was also reported by Kristensen (1972). It is therefore, suggested that the dissolution of style housed in conjoined style sac, is a continuous physical process, taking place throughout the tidal cycle while style secretion is a physiological and rhythmic one, occurring only during the high tide.

The stability of the crystalline style of *D. cuneatus* over a tidal cycle can be attributed to the fact that the style in this species is housed in a style sac completely separated from the midgut. Therefore the style in *D. cuneatus* is isolated from the influence of the gut content as suggested by Yonge (1923). However, the rhythmicity in feeding activity is indicated by the presence and absence of adherent 'mud cap' and food particles on the style. In the case of *D. cuneatus* the rhythm of feeding and digestion may be without corresponding changes in the crystalline style.

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Terminal velocity of the first instar *Ectropis excursaria* (Guenee) (Lepidoptera: Geometridae)

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Abstract. The terminal velocity of first instar *Ectropis excursaria*, determined in a part mechanical part electrical apparatus, were 114 ± 12 cm/s and 118 ± 12·5 cm/s for two different heights. Terminal velocity decreased exponentially with increased silk lengths. For a given length of silk there was a significant difference in the terminal velocity of live and anaesthetised insects. The significance of these results to the understanding of the wind dispersal of first instar caterpillars is discussed.

Keywords. Terminal velocity; *Ectropis excursaria*; first instars; wind dispersal; silk length; anaesthetisation.

1. Introduction

Wind dispersal of the newly emerged first instars is a means of natural spread of many Lepidoptera (Leonard 1974; Mitchell 1979; Batzer 1968; Jennings *et al* 1983), Homoptera (Washburn and Frankie 1981; Moran *et al* 1982) and Acarina (Boykin and Campbell 1984). The distance travelled by a windborne insect depends on the wind velocity, the amount of atmospheric turbulence and the terminal velocity of the larva (McManus and Mason 1983). The determination of terminal velocity is critical to estimating the extent and patterns of dispersal of insects and other particulates (Harrington 1979).

Ectropis excursaria (Geometridae: Lepidoptera) is native to Australia and feeds on several species of Mimosaceae, Myrtaceae and plants of economic importance. The first instars of *E. excursaria* disperse by wind and this dispersal is influenced by host plant species and the quality of foliage of the host plant (Ramachandran 1987). In the present paper, determination of the terminal velocity of the first instars and the influence of silk length and larval activity on terminal velocity is reported.

2. Materials and methods

Live unfed first instars were obtained from a culture maintained on *Eucalyptus camaldulensis* D. Don in an insectary room. The experiment was conducted in an apparatus and the different parts and assembly of which is given in figure 1.

The experimental procedure involved the following steps:

(i) A larva was picked up with a camel's hair brush and induced to spin down on silk by tapping the brush. The tip of the silk was placed on the heating element. The silk invariably stuck to the wire because of its adhesive property. Thus, at the start of the experiment, the insect hung from the heating element such that if its attachment was broken it would fall into the tube.

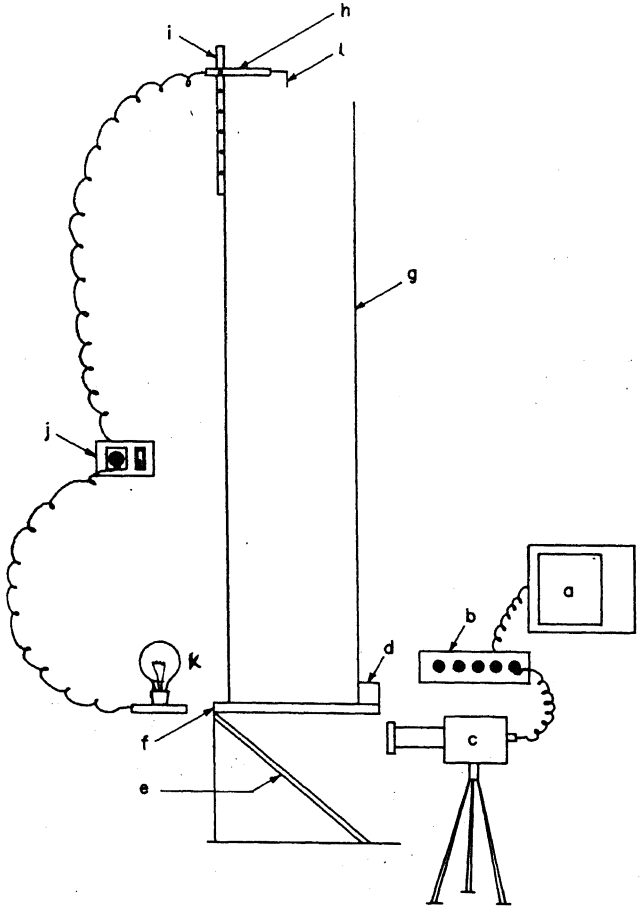

Figure 1. An apparatus for estimating the terminal velocity of insects. **a.** Television monitor. **b.** Video camera recorder. **c.** Video camera. **d.** LED timer. **e.** Mirror. **f.** Glass plate. **g.** Perspex cylinder. **h.** 'Wax melter' used as a insect releaser. **i.** Calibrated rod on which the wax melter slides. **j.** Common power source for insect releaser and light source. **k.** Light source. **l.** A first instar caterpillar hanging on silk from the wax melter.

(ii) By sliding the hot wire along the calibrated scale, such that the insect hung near the rim of the perspex tube, the length of the silk could be measured and recorded.
(iii) The power to the light source and the heating element was switched on.
(iv) At some time after switching on the heating element (the time was recorded manually) the insect was released. In each test, the data on the video recorder allowed determination of 3 stages, (i) when only the digits on the timer were visible i.e. before the start of the experiment, (ii) when the rim of the perspex and the timer were visible i.e. after the light and the heating element had been switched on and (iii) when the timer, rim of the perspex tube and the insect were visible i.e. after the insect had landed on the surface of the glass plate. The time elapsed between each of these 3 events could be calculated from the video tapes by reading the timer and the time recorded in step 4, and hence the time taken by the insect to travel the distance that it had fallen.

The fall velocity of the first instar larvae of uniform size, from 3 heights viz. 100, 160 and 180 cm with a constant silk length of 2 cm was measured in one experiment. In another experiment the fall velocity of larvae with varying silk lengths (1–30 cm) from 100 cm height was recorded. The size of these larvae were measured under a stereo microscope. In a third experiment the time taken to travel 100 cm by live and anaesthetised insects with 2 cm silk was determined. Anaesthetisation was carried out by holding a caterpillar, hanging on silk from the end of the brush, into a beaker containing cotton dipper in chloroform.

3. Results and discussion

The velocity of an object dropped in a fluid medium increases linearly with distance (acceleration phase) to reach a constant value i.e. the terminal velocity. Values of terminal velocity reported by Batzer (1968), Mitchell (1979), Moran *et al* (1982) and McManus and Mason (1983) are the average velocity of the acceleration phase and the terminal velocity phase whereas one strictly requires the period during which the insect travels at terminal velocity. The true terminal velocity was calculated from the experimental fall velocities from the 3 different heights (table 1) in the following manner. For two heights S_1, S_2 (where S_2 is greater than S_1) with times t_1 and t_2, the fall velocities are S_1/t_1 and S_2/t_2. These are average velocities which include an initial phase where the velocity is less than the terminal velocity. If the terminal velocity is approached after a fall of S_1 (the mean distance travelled per second from the 3 different heights did not differ significantly, $F = 0{\cdot}4845$, table 1, suggesting that the terminal velocity might have been reached at less than 100 cm height), the velocity over S_1–S_2 gives an estimate of terminal velocity. The terminal velocity of *E. excursaria* calculated in this manner were $118 \pm 12{\cdot}5$ cm/s for 100–160 cm and 114 ± 12 cm/s for 100–180 cm (from table 1) and were higher than the fall velocities as would be expected.

Although dispersal is the prerogative of winged adults, in insects where the adults are either apterous or do not fly, dispersal is achieved mainly by early first instars. Thus these stages of insects may be expected to show morphological and/or behavioural adaptations that increases the time spent in air and thus the distance travelled from the point of launching. McManus and Mason (1983) reported that the numerous filamentous hairs and long hollow setae in the gypsy moth first instar larvae might increase the buoyancy of these wind dispersing caterpillars. The production of silk by wind dispersing apterous insects (lepidopteran hatchlings and arachnids) is considered to reduce their terminal velocity (Batzer 1968; Mitchell 1979;

Table 1. Fall velocities of the first instar caterpillars dropped from different heights.

Height	Larval condition	n	Fall velocity ± SEM
100	Live	28	$108 \pm 2{\cdot}3$
100	Anaesthetised	25	$114 \pm 1{\cdot}6$
160	Live	32	$111 \pm 2{\cdot}4$
180	Live	13	$110 \pm 5{\cdot}0$

McManus and Mason 1983). In the present studies the effect of silk length of upto 30 cm on the fall velocity of the first instars was determined. The relationship between the length of silk and fall velocity showed an exponential decrease and the equation $Y = 153{\cdot}7\ X^{-0{\cdot}507}$ (in its logarithmic form) was fitted to the data (figure 2). A similar exponential decrease in the effect of silk length on the fall velocity of airborne caterpillars has been reported by Batzer (1968) and Mitchell (1979). The relationship suggests that there may be an optimal length of silk (somewhere between 15–25 cm) above which the increase in the time spent in air due to additional silk length is offset by the risk of the silk being caught on objects near the point of launching and the energetics of silk production. Indeed Batzer (1968) observed that 79% of spruce budworm larvae produced less than 30 cm of silk on initial disturbance.

McManus and Mason (1983) showed a linear increase in terminal velocity with the range of weight of unfed first instar gypsy moth. Size of the insect would also have an important effect on the terminal velocity as the drag force depends on the size and shape of the object. To this end, when the experiment on the effect of silk length was conducted, the length of the insects were measured. A multiple regression of the time taken to travel one meter to the silk length and the length of the insect indicated that the range of sizes tested explained only 0·2% of the observed variation, while silk length explained about 84·5% of the variation (regression of silk length to terminal velocity significant ANOVA, $F = 387{\cdot}10$, $P < 0{\cdot}001$; regression of size of the insect to terminal velocity not significant ANOVA, $F = 1{\cdot}96$). The first instars used in the experiment ranged in size from 1·40–2·5 mm, with a mean ± SD of 1·97 ± 0·24 mm

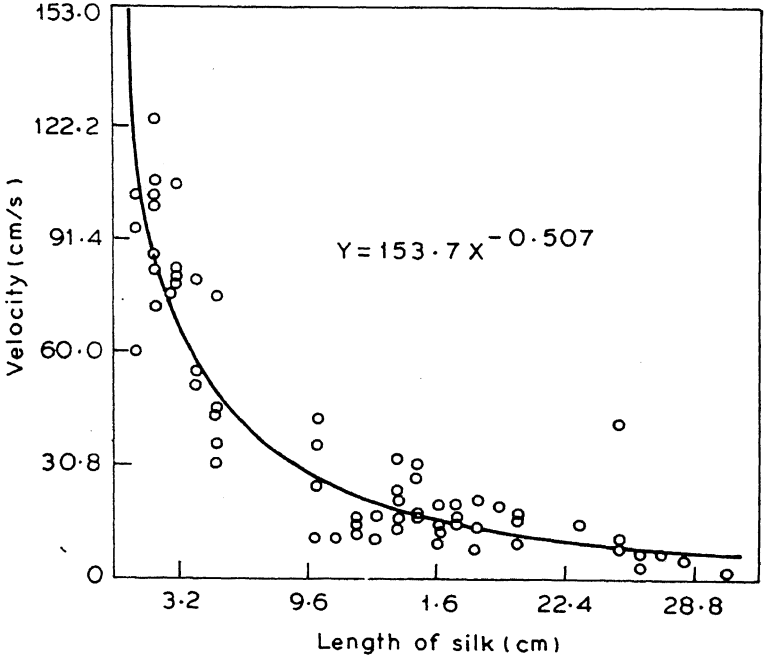

Figure 2. Relationship between the length of silk attached to the caterpillar and the terminal velocity of first instars of *E. excursaria*.

(n = 71). This size range is probably not enough to exert any significant influence on terminal velocity.

Anaesthetisation of caterpillars significantly increased the fall velocity as compared to live caterpillars ($t_{51} = 2{\cdot}61$, $P < 0{\cdot}05$, table 1). Similar differences in the terminal velocity of live and dead wingless first instars of coccids (*Pulvinarella mesembryanthemi*) were reported by Washburn and Washburn (1984). They have reported that live coccids arched their body backwards and extended their legs and antennae outwards while freshly killed ones fell almost like a sphere. In flightless grasshoppers, jumps from elevated positions followed by aerobatic manoeuvring has been shown to carry them several metres through the air (Cohn and Cantrall 1979; Arbas 1983). Live first instars of *E. excursaria* also seem to exhibit adaptive behaviour such as assuming a posture or flexing their body to reduce the terminal velocity and enable them to stay in the air longer. However, further evidence for such aerial reflexes can only be obtained by photographing the dispersing insects.

Mason and McManus (1979) incorporated the values of terminal velocity into an atmospheric dispersion model to predict the distance dispersed by first instar gypsy moth. Based on the results they concluded that most of the dispersal of first instars were only short range. The terminal velocity of *E. excursaria* is higher than that of gypsy moth (70–110 cm/s, McManus and Mason 1983) suggesting that wind dispersal in this insect also leads to only short distance movement. However, results of the present investigation that silk length and larval activity could reduce the terminal velocity of the larvae indicate possibilities of longer distance dispersal by an interaction of factors such as silk length attached to the caterpillar, larval activity in air and the nature and velocity of wind.

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Evaluation of brodifacoum against house murids of Garhwal Himalaya (India)

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Abstract. Brodifacoum (WBA 8119) was tested at various concentrations (0·02, 0·01, 0·005, 0·0025 and 0·00125%) by choice tests in laboratory against house murids viz *Rattus rattus gangutrianus* Hinton and *Mus musculus* Linnaeus of Garhwal Himalaya. It was fairly acceptable and produced 100% mortality of both the target species at all levels of its concentrations except at 0·00125% level causing 83·3% mortality in *Rattus rattus gangutrianus* and 100% in *Mus musculus*. The mean period of mortality is increased with the decrease in the concentration, the range being 3–17 days. In house trial a short exposure of 1-night of moist brodifacoum (0·0025%) bait after pre-baiting for 3-nights produced 100% murids mortality which was significantly ($P<0·01$) higher than that achieved in a similar trial with dry brodifacoum bait.

Keywords. Brodifacoum; murids; *Rattus rattus*; *Mus musculus*; bait; pre-baiting.

1. Introduction

A new anticoagulant, brodifacoum (3-(3–4-bromobiphenyl-4-yl)-1, 3–4-tetrahydrol-naphthyl)-4-hydroxy-coumarin) showed promising rodenticidal properties producing 100% mortality in various rodent species at its 0·005% concentration (Hadler and Shadbolt 1975; Dubock and Kaukeinen 1978; Dubock 1980; Soni and Prakash 1984; Parshad *et al* 1985). The acceptability and the efficacy of this compound against house murids viz. *Rattus rattus gangutrianus* Hinton and *Mus musculus* Linnaeus in a different climatic zone of Garhwal Himalaya (long. 78°–47′E, lat. 30°–13′N and Alt. 570 m) are reported in this paper.

2. Materials and methods

For laboratory evaluation of the compound 3 male and 3 female healthy animals of *R. r. gangutrianus* and *M. musculus* were individually caged for each set of experiments and acclimatized for 15 days before the start of experiments. The compound was tested at 0·02, 0·01, 0·005, 0·0025 and 0·00125% concentration by choice-test. Dry '*koda*' (*Eleucine coracana*) flour was used as rodenticide carrier. Treated bait was exposed for 48 h in one of the food cups and simultaneously untreated food was presented in the other food cup. In the pre- and post-treatments daily consumptions of food (untreated '*koda*' flour) were also recorded and water was provided *ad libitum* throughout the experiment.

For house trials separate sets of 5 houses (replicates) were treated with 0·0025% brodifacoum in dry '*jhangora*' (*Echinocloa frumentacea*) grains (trial-H1) and moist boiled '*jhangora*' (trial-H2). Bait was exposed for 1-night from 10 pm to 6 am. The

pre- and post-treatment censuses were carried out by placing dry '*jhangora*' grain in trial-H1 and moist boiled '*jhangora*' in trial-H2 for 1-night after pre-baiting for 2-nights. The pre-treatment census baiting also functioned as pre-baiting for the treatment. The mortality of murids in houses was calculated from the weights of census bait eaten, as follows (Handerson and Tilton 1955).

$$\text{Mortality } (\%) = 100 \left(1 - \frac{t_2 \times r_1}{t_1 \times r_2}\right),$$

t, census bait take from treated house; r, census bait take from reference house; t_1, r_1, pre-treatment census bait takes; t_2, r_2, post-treatment census bait takes.

3. Results and discussion

Both the murids consumed statistically equal amounts of '*koda*' flour ($P<0{\cdot}05$). However, when 0·02% brodifacoum was used in bait, *R. r. gangutrianus* consumed significantly ($P<0{\cdot}05$) more poison bait than plain food (table 1). The higher acceptance of 0·02% brodifacoum bait over plain food might be due to the presence of large amount of sugar content furnished by sugar-based liquid concentrate of the compound. The daily food consumption, on treatment days and 1 day after treatment differed non-significantly with the pre-treatment normal food consumption (table 1). These results signify an absence of poison aversion and bait shyness. Evidently ill effect of treatment did not appear till 1 day after treatment. Nevertheless, many workers have suggested that the brodifacoum bait was fairly acceptable on first day but thereafter acceptability declined (Soni and Prakash 1984; Parshad *et al* 1985).

In the laboratory conditions brodifacoum showed promising efficacy against *R. r. gangutrianus* producing 100% mortality at all concentrations tested except at 0·00125% level when the kill was 83·33%. Present *R. rattus* appeared to be more susceptible to brodifacoum than reported from other ecological regions (Redfern *et al* 1976; Dubock and Kaukeinen 1978; Parshad *et al* 1985). In the present study, *M. musculus* proved to be more susceptible to the chemical as its mortality was 100% even at 0·00125% concentration. The higher susceptibility of *M. musculus* over *R. rattus* to brodifacoum is also evident from their acute oral LD_{50}, 0·40 mg/kg body weight (Redfern *et al* 1976), and 0·65 mg/kg body weight (Dubock and Kaukeinen 1978) respectively. Efficacy of brodifacoum against *M. musculus* is very much similar to the findings of Dubock and Kaukeinen (1978). In contrast a high degree of innate tolerance of brodifacoum has been observed in *M. musculus* of the Thar desert (Soni and Prakash 1984). The mean periods of mortality after treatment were observed to increase with the decrease in the concentration of rodenticide in the bait (table 1). The periods of mortality appeared to be linked with the amount of active ingradient ingested by the rodents.

In the houses of the present study, 1-night exposure of moist brodifacoum bait produced significantly ($P<0{\cdot}01$) higher rodent mortality than that of the dry rodenticidal bait (table 2). In reference houses moist census bait consumption was almost triple to that of dry alternative (table 2). Therefore, significant higher efficacy of moist bait of brodifacoum over dry one (table 2) can be attributed to its high acceptability. Siddiqui and Khan (1982) have also observed that moist baits are more

Table 1. Acceptability and efficacy of brodifacoum baits in laboratory.

Species	Dose of brodifacoum (%)	Consumption in g/100 g body weight (Mean ± SE)						
		Pre-treatment daily consumption a	Treatment (2-days)		Daily consumption on treatment days b	Plain bait 1 day after treatment c	Consumption of brodifacoum in 48 h mg/kg body weight*	Time taken to death (days) Mean (range)
			Brodifacoum bait b_1	Plain food b_2				
R. r. gangutrianus	0·02	7·65 ± 0·27	8·24 ± 0·20	7·14 ± 0·28	7·68 ± 0·10	7·77 ± 0·67	$16·53 \pm 0·42^{A}$	4·33 (3–4)
	0·01	8·12 ± 0·33	8·44 ± 0·38	7·6 ± 0·32	8·05 ± 0·17	7·83 ± 0·46	$8·52 \pm 0·38^{B}$	5·00 (4–6)
	0·005	8·03 ± 0·68	8·06 ± 0·42	7·90 ± 0·38	7·90 ± 0·33	8·95 ± 0·24	$4·06 \pm 0·21^{C}$	5·17 (4–7)
	0·0025	7·98 ± 0·27	7·56 ± 0·40	7·94 ± 0·34	7·7 ± 0·22	7·85 ± 0·31	$1·90 \pm 0·10^{D}$	8·17 (6–10)
	0·00125**	7·53 ± 0·08	7·40 ± 0·24	7·46 ± 0·42	7·40 ± 0·20	7·58 ± 0·43	$0·94 \pm 0·30^{E}$	10·40 (8–12)
M. musculus	0·0025	10·18 ± 0·24	10·20 ± 0·48	9·46 ± 0·28	9·83 ± 0·11	10·07 ± 0·73	$2·56 \pm 0·12^{A}$	7·70 (4–11)
	0·00125	11·03 ± 0·41	10·78 ± 0·58	10·90 ± 0·72	10·83 ± 0·19	11·18 ± 0·28	$1·35 \pm 0·07^{B}$	9·20 (4–16)

Mean consumptions b_1 vs b_2, a vs b and a vs c differ non-significantly in all the corresponding dose except that at 0·02% dose b_1 vs b_2 differ significantly at 1% level.

*Ranks A, B, etc. are given on the basis of critical differences for *R. rattus* (0·734) and *M. musculus* (0·342) at 5% level; **brodifacoum produced 100% mortality of both the murid species at all the concentration except that at 0·00125% concentration mortality of *R. rattus* was 83·33%.

Table 2. Results of brodifacoum baiting in residential premises.

Trial	Treatment	Pre-treatment census bait g/night	Post-treatment census bait g/night	Rodent mortality (%)
H1	Brodifacoum dry bait	57·40 ± 9·33	32·80 ± 5·84	50·17 ± 2·22[D]
H2	Brodifacoum moist bait	182·20 ± 9·63	0·00 ± 0·00	100·00 ± 0·00[C]
Reference:	Rodenticide free food			
	Dry	51·40 ± 1·54[B]	59·20 ± 3·21	+ 15·17
	Moist	161·80 ± 6·89[A]	166·60 ± 4·80	+ 2·97

±, Show SE of means; +, shows increase in rodent population; A vs B and C vs D differ significantly at 1% level.

acceptable to rodents. The high rodent mortality after 1-night baiting of moist brodifacoum bait holds promise for its use in the residential premises. The short exposure of moist brodifacoum bait after 3-days pre-baiting with plain food (moist) reduces the poisoning hazards to children and pets, making the control operation more acceptable by the people.

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Qualitative and quantitative changes in lipids along the length of female reproductive system of the poultry nematode *Ascaridia galli* (Schrank 1788)

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Abstract. Qualitative and quantitative studies have been made of lipids along the length of the female reproductive system of the poultry nematode, *Ascaridia galli*. Adult specimens were collected in 0·9% saline from the intestines of naturally infected fowls. The quantitative studies have revealed significant ($P = 0{\cdot}05$) differences of total lipids, phospholipids, sterols, free fatty acids and glycerides and significantly ($P < 0{\cdot}001$) higher amounts of non polar than polar lipids in different regions of the reproductive system. The qualitative studies have showed that the germinal, growth and maturation regions of the ovary, oviduct and uterus contained 7, 7, 6, 6, 10 fractions respectively of polar and 12, 12, 12, 11, 10 fractions respectively of non polar lipids.

Keywords. *Ascaridia galli*; lipids; nematode; ovary; oviduct; oogenesis; uterus.

1. Introduction

The female reproductive system of ascarid nematodes contains high amounts of lipids (Fairbairn 1955, 1957). These lipids undergo marked changes in their amount and localization along with the length of *Ascaridia galli* (Schrank 1788) as revealed by histochemical techniques (Parshad and Guraya 1982). But no detailed quantitative and qualitative studies have been made previously of lipids during oogenesis of nematodes. Their aspect in germinal, growth and maturation regions of the ovary, oviduct and uterus have been studied for the poultry nematode *Ascaridia galli*. The possible physiological significance of various lipid changes is also discussed.

2. Materials and methods

The adult specimens of *A. galli* were collected in 0·9% saline from the intestines of naturally infected fowls, *Gallus domesticus*. The worms were cut longitudinally and their isolated reproductive systems were divided into germinal, growth and maturation regions of the ovary, oviduct and uterus. Confirmation of these regions of the reproductive system was based on their histological examination (Parshad and Guraya 1982). The different regions of the female reproductive system from a large number of worms were pooled separately and subjected to the extraction of total lipids in chloroform: methanol (2:1) (Folch *et al* 1957). For the quantitative determination of sterols, free fatty acids and phospholipids, the methods of Stadtman (1957), Mahadevan *et al* (1969) and Ames (1966) were used respectively. The remaining part of the lipids was considered to be the glycerides. The difference between the lipid fractions of various regions were compared by student's '*t*' test and analysis of variance.

Fractionation of lipids was carried out by thin layer chromatography (TLC) using chloroform; methanol: 7N NH_3 (75:25:4) for non polar (Horrocks 1963) and petroleum ether:ethyl ether:acetic acid (80:20:1) for polar lipids (Williams *et al* 1960). The colouring reagents used were iodine vapours (Sims and Larose 1962), sulphuric acid (Heftman *et al* 1966) and cupric acetate phosphoric reagent (Fewster *et al* 1969). The different fractions have been identified from their R_f values (Work and Work 1972).

3. Results

The amounts of total lipids, phospholipids, sterols and free fatty acids of the ovary were significantly ($P=0.05$) more and of glycerides less than that of the uterus (table 1). The oviduct contained significantly ($P=0.05$) more amounts of total lipids and free fatty acids than any other part of the female reproductive system (table 1). The female system contained significantly ($P<0.001$) higher amounts of non-polar than the polar lipids (table 2). The germinal, growth and maturation regions of the ovary contained significantly ($P=0.05$) different amounts of total lipids, sterols and free fatty acids. The amount of phospholipids of growth and maturation regions did not differ significantly but was significantly ($P=0.05$) less than that of the germinal region. The amount of glycerides of the germinal and growth regions, was significantly ($P=0.05$) less than that of the maturation region.

The germinal, growth and maturation regions of the ovary, oviduct and uterus contained 7, 7, 6, 6, 10 fractions respectively of polar lipids (table 3) and 12, 12, 12, 11, 10 fractions respectively of non-polar lipids (table 4).

Table 1. Lipid composition ($\bar{x} \pm SE$) of the female reproductive systems of *A. galli* (mg/100 mg tissue on wet weight basis).

Parts of reproductive system	Amount of lipids				
	Total lipids	Phospholipids	Glycerides	Sterols	Free-fatty acids
Ovary	6·60 ± 0·04	1·51 ± 0·11 (22·88)	2·44 ± 0·03 (36·97)	1·43 ± 0·006 (21·67)	1·22 ± 0·01 (18·48)
Germinal	7·16 ± 0·06	1·89 ± 0·01 (26·39)	2·89 ± 0·06 (40·36)	1·17 ± 0·02 (16·34)	1·20 ± 0·01 (17·76)
Growth	8·10 ± 0·08	1·27 ± 0·04 (15·68)	2·53 ± 0·13 (31·23)	2·49 ± 0·02 (30·74)	1·80 ± 0·02 (22·22)
Maturation	4·54 ± 0·10	1·36 ± 0·01 (29·96)	1·89 ± 0·11 (41·63)	0·63 ± 0·002 (13·88)	0·66 ± 0·01 (14·57)
Oviduct	18·00 ± 0·08	NR	NR	NR	4·07 ± 0·01 (22·61)
Uterus	5·56 ± 0·12	0·35 ± 0·01 (6·9)	4·29 ± 0·12 (77·16)	0·47 ± 0·01 (8·45)	0·45 ± 0·01 (8·09)
CD* ($P=0.05$) n = 5	0·2794	0·0917	0·3803	0·0583	0·0201
CD** ($P=0.05$) n = 5	0·3025	0·0347	0·3867	0·0408	0·1021

Numbers in parentheses represent percentages of the respective fractions with respect to total lipids.
NR, Not recorded.
*CD between different regions of the ovary.
**CD between different parts of the reproductive system.

Table 2. Non-polar and polar lipid composition ($\bar{x} \pm SE$) of the female reproductive system of *A. galli* (mg/100 mg tissues of wet weight basis).

Parts of reproductive system	Amount of lipids		*t*-test between non-polar and polar lipids	
	Non-polar	Polar		
Germinal	5·26 ± 0·07	1·89 ± 0·01	$t_8 = 48{\cdot}44$	$P < 0{\cdot}001$
Growth	6·82 ± 0·09	1·27 ± 0·04	$t_8 = 54{\cdot}85$	$P < 0{\cdot}001$
Maturation	3·18 ± 0·01	1·36 ± 0·01	$t_8 = 17{\cdot}99$	$P < 0{\cdot}001$
Uterus	5·20 ± 0·13	0·35 ± 0·001	$t_8 = 38{\cdot}34$	$P < 0{\cdot}001$

Table 3. Polar lipids of female reproductive system of *A. galli*.

Spot No.	Regions of ovary					Inference
	Germinal	Growth	Maturation	Oviduct	Uterus	
1	+	+	−	−	+	Phosphatidyl serine, phosphatidic acid, lysophosphatidic acid
2	−	−	+	+	−	Lysolecithin
3	+	+	+	+	+	Phosphatidyl inositol
4	−	−	+	+	+	Cerebroside sulphate
5	+	+	+	−	+	Sphingomyelin
6	−	−	+	+	−	Unidentified
7	−	−	−	−	+	Lecithin
8	−	−	−	−	+	Phosphatidyl ethanolamine
9	−	−	−	−	+	Cerebrosides
10	+	+	−	−	+	Unidentified
11	+	+	+	+	+	—"—
12	+	+	−	+	+	—"—
13	+	+	−	−	−	—"—

Table 4. Non-polar lipids of female reproductive system of *A. galli*.

Spot No.	Regions of ovary					Inference
	Germinal	Growth	Maturation	Oviduct	Uterus	
1	+	+	+	+	+	Monoglycerides
2	+	+	+	+	+	1–0-Monoalkyl glycerol ethers
3	+	+	+	−	+	Unidentified
4	+	+	+	−	+	—"—
5	−	−	−	+	−	—"—
6	+	+	+	+	+	1,2-Diglycerides
7	+	+	+	+	+	Sterols
8	+	+	+	+	−	1,3-Diglycerides
9	+	+	+	+	−	0-Dialkyl glycerol ethers
10	+	+	+	+	+	Fatty acids
11	+	+	+	+	+	Triglycerides
12	+	+	+	+	+	Aldehyde dimethyl acetals/long chain aldehydes
13	+	+	−	−	−	Alk-1-enyl diglycerides
14	−	−	−	−	+	Tri-alkyglycerol ethers
15	−	−	+	+	−	0-Dialkyl monoglycerates

4. Discussion

The amount of total lipids of the female reproductive system of *A. galli* estimated quantitatively ranged from 5·56 ± 0·12% in the uterus to 18 ± 0·08% in the oviduct. These studies as well as Fairbairn (1955), Lee (1960), Parshad and Guraya (1982) and Frayha and Smith (1983) have clearly revealed that the nematode female reproductive system is an important site for accumulation of large amount of lipids, which are utilized in egg production (Krusberg *et al* 1973). This is supported by the observation that the enzymes of β-oxidation are present in embryonating eggs of *Ascaris* sp. (Barrett *et al* 1970; Ward and Fairbairn 1970). The morphology, distribution and the amount of lipid inclusions showed progressive increase in the oocytes from the anterior germinal to the growth zone of the ovary (Anya 1964; Parshad and Guraya 1982). The mature ova contained largest amount of lipids. The present biochemical estimates of the total lipids of the germinal and growth regions of the ovary agreed with the previous histochemical findings but differed in case of the maturation region.

The amount of non-polar lipids of all the regions of the ovary of *A. galli* was significantly ($P = 0{\cdot}001$) more than the polar lipids. The female reproductive systems of nematodes contained greater amount of neutral lipids which served as substrates for carbohydrate synthesis in the developing embryos (Fairbairn 1955; Tarr 1972). The growth region of the ovary of *A. galli* contained the maximum amount of sterols which might be used for the development of cellular organelles in growing oocytes (Krusberg 1967; Hirsch and Rothstein 1968; Barrett *et al* 1970) along with phospholipids in synthesis of cellular and subcellular membranes (Fairbairn 1957).

The growth region of the ovary of *A. galli* contained maximum amount of free fatty acids which decreased in the maturation region. Possibly larger amounts of free fatty acids are utilized in the synthesis of ascaroside esters in the oocytes. In the maturation region of the *Ascaris* almost all of the ascarosides of the maturing oocytes get esterified and these esters possibly form the precursors of the ascaroside layer of the egg shell (Fairbairn and Passey 1955; Wharton 1980).

The qualitative differences of the polar and non-polar lipid fractions between the ovary and the uterus are attributed to the different metabolic needs of the oocytes and the eggs.

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Stress induced alterations in the hemocyte population of *Periplaneta americana* (L.)

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Abstract. Alterations in the hemocyte population of *Periplaneta americana* (L.) during starvation and desiccation were studied for 11 days. Total hemocyte count showed increasing trend throughout the starvation period. Changes in differential hemocyte count were evident. Plasmatocytes and granulocytes decreased marginally. Increase in coagulocyte count occurred from 6th day onwards. Two-fold increase in the total hemocyte and differential count was pronounced during subsequent days. During the combined stress period total hemocyte count was significantly higher. Differential hemocyte count was also altered. Degenerative changes set in during late phases of the experiment. The causal factors for such changes in hemocyte population under experimental stress are discussed.

Keywords. Roach; *Periplaneta americana*; hemocytes; desiccation; starvation; total and differential counts.

1. Introduction

Knowledge of the insect hemocyte population is essential for physiological and other studies. Data on total and differential counts in insects are available. The hemocyte counts vary during development, physiological status, starvation, wounding, infection etc. (Jones and Tauber 1952; Shapiro 1966, 1967, 1968, 1979; Jones 1967; Arnold and Hinks 1976; Gupta 1985; Sonawane 1985). Inadequate information on starvation and almost no information on desiccation induced changes in insect total hemocyte count (THC) and differential hemocyte count (DHC) are available. The above stresses are known to induce certain physiological changes. Present work is aimed at finding out the effects of the above two metabolic stresses, separately and in combination, on THC and DHC of *Periplaneta americana.*

2. Material and methods

Adult roaches of the same age and with no regard to sex, were collected from a single den. Four batches, each of 50 roaches were used for the experiments. Batch I served as control. It was kept under laboratory conditions. For starvation, roaches of batch II were employed. They were supplied with water but no food. Batch III was exposed to desiccation by keeping the roaches in plastic containers having a number of holes in them. Such containers were kept in desiccators containing anhydrous $CaCl_2$. The roaches were, however, supplied with food. For combined effect, the roaches (batch IV) were kept as above and to them neither food nor water was supplied.

The THC and DHC of the roaches of the above batches were done every 24 h, for 11 days. All the roaches were found dead on 12th day. The hemolymph was obtained

by amputation of antennae. After dilution (in Thoma-Zeiss hemocytometer) the hemocytes were counted in Neubaur's hemocytometer (Witting 1966). Following formula of Jones (1962) was adopted for calculations.

$$\frac{\text{Hemocytes in} \times 1\,\text{mm squares} \times \text{dilution} \times \text{depth of the chamber}}{\text{Number of 1 mm squares counted}}$$

For the DHC, fixed stained monolayers (Sonawane 1985) were used.

3. Results

3.1 *Starvation*

During the entire starvation period the THC remained higher (control, 22, 291 ± 262). On 5th day the count was the highest (40, 935 ± 1067) and on the termination day it was 32,031 ± 692 (table 1).

Prohemocytes (PRS) increased to 6% (control, 3%) after 2 days of starvation. Percentage of plasmatocytes (PLS) and granulocytes (GRS) decreased marginally. No change in the count was seen in the coagulocytes (COS) and spherulocytes (SPS). On the 3rd day PLS increased to 65% (control, 53%). Amongst them the spindle shaped were dominating. They were almost more than half the total number of PLS. On the 5th day PLS decreased to 45% and the GRS increased in their count to 60% (control, 40%). At this stage, even the PLS contained granules which were basophilic and hence similar to the granules in GRS seen under normal conditions. Increase in the COS count commenced from the 6th onwards (control, 3%). On the 7th day their count was 5·5%. From the 8th day onwards distinct morphological changes were

Table 1. Effect of starvation, desiccation and starvation and desiccation (combined) on THCs of *P. americana.*

Experimental period (days)	Experimental condition (THC, mean ± SE)		
	Starvation	Desiccation	Starvation and desiccation
1	—	—	—
2	36,250 ± 883	42,500 ± 2,041	60,062 ± 1,662
3	38,125 ± 255	25,312 ± 598	62,406 ± 487
4	38,750 ± 510	22,500 ± 510	88,687 ± 514
5	40,935 ± 1,067	91,000 ± 4,102	62,375 ± 625
6	35,625 ± 555	74,437 ± 1,165	56,125 ± 1,586
7	34,343 ± 257	68,093 ± 2,634	54,000 ± 797
8	32,968 ± 1,032	27,500 ± 2,041	63,750 ± 510
9	33,656 ± 271	11,093 ± 553	57,562 ± 482
10	33,843 ± 180	8,875 ± 525	44,487 ± 1,067
11	32,031 ± 692	—	52,031 ± 781

Mean THC of control insects was 22,291 ± 261.
Differential hemocyte count of control insects was PRS, 3%; PLS, 53%; GRS, 40%; SPS, 1%; COS, 3%.

evident in some of the GRS. In some such cells, the granules were pushed towards the periphery. The nuclear volume showed increase. Their chromatin was loosened and dispersed. Perinuclear cisternae were quite evident in them. The GRS remained the dominating hemocyte type from 5th onwards. They were followed in number by PLS, COS, PRS and SPS in that order.

On the 10th day the count of different hemocytes was GRS 56%, PLS 30%, COS 6%, PRS 4·5% and SPS 3·5%.

3.2 *Desiccation*

Alterations in THC during desiccation are presented in table 1. Two-fold increase in the count occurred during the first two days. On the 4th day it appeared to be normal. There was an abrupt increase (91,000 ± 4102) on the 5th day. The count started decreasing from the 6th day onwards. On the 10th day it was 8,875 ± 825. Most of the PLS observed on the 3rd day were spindle shaped. They became polymorphic on the 4th and 5th day. The DHC remained unchanged upto the 8th day. From this day onwards, the degenerative changes commenced in all the hemocyte types. Such changes were frequent and pronounced in the GRS. Because of this, some of them resembled the COS. Cell fragmentation was rapid on the 10th day and it was impossible to distinguish the cell types on subsequent days.

3.3 *Starvation and desiccation*

The THC was significantly higher in *P. americana* during the above combined stress period. Data in table 1 reveal the highest count (88,687 ± 514) on the 4th day and minimum on the 10th day (44,487 ± 1067). The data also indicate that the fluctuations in the total hemocyte count occur but they are, however, of a smaller magnitude than during the stress conditions applied separately.

After two days, the GRS predominated. Together with the PLS they comprised the main bulk on the 4th day (80–89%). The PRS increased to about 7·10%, the COS about 3% and the SPS less than 1%. Increase in the COS was evident on the 5th day (about 6%). At the end of the 7th day, however, the COS percentage dropped below average (i.e. less than 3%). Degenerative changes in many PLS and the GRS were initiated by the end of the 8th day. Their cell membrane was inconsistent, ill defined, and lysed at many places. Enhanced degenerative changes were evident from the 9th day onwards till the termination of the experiment. On the 11th day, surprisingly, some newly formed PLS and GRS were seen along with the old degenerating hemocytes.

4. Discussion

Jones (1950) reported that no appreciable change was seen in the THC of the last instar larvae of *Tenebrio molitor* starved for 30 days. According to him there exists a tendency in them for the GRS to increase and the PLS to decrease in count. Apparent hemocytopenia sets in after starvation for 120 days. During this period the PRS increased many folds. Decrease in the THC is evident after prolonged starvation

(Jones and Tauber 1952) in the same insect. An increase in the THC is reported in *Leptinotarsa* (Arvy *et al* 1948) and *Prodenia* (Yeager 1945; Rosenberger and Jones 1960) during starvation. On the other hand a decrease in the THC is seen in the starved larvae of *Bombyx mori* (Nittono 1960) and in *Galleria* (Shapiro 1966). From the above it is apparent that in some insects there is an increase and in others decrease in the THC occurs following starvation. For the above changes in hemocyte population, variations in the blood volume (BV) are thought to be responsible. According to Arvy *et al* (1948) and Rosenberger and Jones (1960) the BV decreases during starvation. Observations made by Yeager and Munson (1950) on *P. americana* indicate no change in the BV. It is well known that under adverse conditions and at the time of experimental stress, particularly when metabolic water is not available, fluid from the tissue spaces is added to the circulating hemolymph (Shapiro 1979; Gupta 1985), thus increasing the hemocytes.

Increase in the hemocyte population has been attributed to the mitotic activity. Throughout the experiment no mitotic divisions were seen. So this reasoning fails to satisfy the reason for the increased THC.

Another possible reason for the increase in the THC is well argued by Shapiro (1966, 1979). According to him the mitotic activity (if at all it exists during starvation) would not be solely responsible for the increase in THC. However, he is of the view that the existing transitional cells differentiate into various types. In addition, the hemocytes adhering to the tissue and organ surfaces are brought into circulation which leads to increase in the THC. This view is supported by Jones (1962), Shapiro (1979) and Gupta (1985). Logically this argument seems to be acceptable for the present results. During the last phase of the present experiment degenerative processes set in and hence the THC decreased during the last phase of starvation.

An outbreak of the PLS on the 3rd day, domination of the GRS after the 5th day and increase in the COS count from the 6th day onwards suggest progressive transformation of one hemocyte type into another. This view is adequately substantiated (Shapiro 1979; Gupta 1985) for normal and experimental conditions. It has been shown that the sessile PLS enter the circulation during the experimental stress conditions and hormonal stimulation (Pathak 1983). The same PLS differentiate further into GRS. This entire process leads to the increase in THC under such situations. This view has been also put forward by Pathak (1983) and he has shown that the THC of *Halys dentata* alters under the influence of neurosecretory cells and corpora cardiaca. Decrease in the THC after the 8th day is due to the degenerative changes in the hemocytes. The GRS seem to be involved in the intermediary metabolism and the metabolism is directly or indirectly under the hormonal control (Wigglesworth 1979).

Fluctuations in the THC during desiccation most probably reflect changes in BV. As the DHC did not change, it seems obviously that desiccation during the first two days probably reduces the BV and hence, there is a rise in the THC. In the next two days some mechanism (endocrine?) might be operating to try to regulate the BV. Drastic desiccation at the end of the 5th day might not be allowing the BV to make up itself, and this might have resulted in an abrupt increase in THC. Decrease in the THC during the last phase is because of the degenerative and lytic processes. Unfortunately there are no comparable data in this regard, hence, at this juncture it is not appropriate to draw definite conclusions.

4. Discussion

Wilcoxon and Hartzell (1933) observed vacuolation and trigrolysis of the Nisl granules and tissue disintegration in the brain and ganglia of the ventral nerve cor and to some extent in the connectives also in the larvae of *Tenebrio molitor* afte treatment with pyrethrum. Klinger (1936) in the larvae of gypsy moth, *Porthetri dispar*, observed that a high dose of pyrethrum, 24 h after application, cause extensive damage to the nerve cells of the abdominal ganglia, especially towards th periphery. On the other hand, a number of well known insecticides do not produc any visible histopathological changes in insects. Kruger (1931) and Hartzell (1934 failed to find any effect on the central nervous system by applying rotenone. Thus i is evident that the mode of action and effects of different insecticides is not uniform i all the insects.

It is also known that the neurilemma of the insect central nervous system plays role in the transport of nutrients in the nervous system (see Chandra and Singl 1982), assists to control the movements of ions into and out of the nervous systen (Hoyle 1953; Pichon *et al* 1972; Lane 1972; Schofield and Treherne 1978) an performs an osmoregulatory function (Lorentè de No 1952; Shanes 1953). Lik nutrients, the insecticides also might be entering through the neurilemma, perineu rium and ultimately reaching to the glial cells, neurons and axons. The presence c bound lipid nerve sheath and their probable relation to the penetration of neurotoxi insecticides is of major interest in any study of mode of action of these toxins. Mos of the earlier workers reported the destruction of this sheath by the insecticida action thus facilitating the insecticides entry, but we do not find this type c destruction after the treatments with sublethal doses of dichlorvos and carbaryl o *P. ricini*. Thus there may be two possibilities for the entrance of these chemical inside the cortex of the central nervous system of the insects—(i) either the entir lipid sheath, covering the nervous system degenerates or (ii) the insecticides diffus through the tissue barriers (connective tissue sheath, neurilemma, perineurium an cortex). The former type of insecticide entry is reported by Klinger (1936), Richard (1941, in mosquito larva) and Wigglesworth (1941). Shaheen and Singh (1986) i *Diacrisia obliqua* and in the present study found cellular destruction in treated insect but did not include the destruction of the nerve sheath. This may be possible tha either lower doses of these chemicals do not cause sheath destruction or that thi change is insecticide specific.

Insecticides induce the division of cells. An enormous cell death due to insecticida actions is most probably compensated by this increased cell division. The increase i the mitotic figures in treated insects might be due to this reason. The larval neura lamella is retained for a maximum period. In normal development it is degenerate in the early pupal period but in the treated insects it is retained till the early adu stage. It indicates that the treated insects try to retain the neural lamella as far a possible to avoid the entry of the chemical(s) present in the haemolymph. Othe morphogenetic changes and differentiation of various nervous centres are no affected by the insecticidal treatment because the metamorphic changes in the centra complex, optic ganglia and deutocerebrum etc take place normally and timely.

Treatments with dichlorvos and carbaryl produce vacuoles in the brain and gang lia. Clumping of chromatin, disintegration of cellular components and neurite swelling and deformities in the nerve cells have also been noted. Particle formation i

so noted (Richards and Cutkomp 1945; Shaheen and Singh 1986). Appearance of acuoles in the perineurium is also noted which may be due to the neuronal death used by insecticidal action.

On the basis of the present study we have come to the following conclusions: Treatments with dichlorvos and carbaryl do not interfere with the normal development, morphogenetic changes and differentiation of various centres of the brain nd ganglia, but cellular and fibre disintegration of various degrees occurs, (ii) that eated insects try to retain the larval neural lamella for a maximum period and i) that insecticides probably induce cell division.

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Effect of cruciferous glucosinolates on probing pattern and feed uptake by mustard aphid, *Lipaphis erysimi* (Kaltenbach)

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Abstract. The glucosinolate content of the 3 host plants, viz. yellow *sarson*, *Brassica campestris* L. var. yellow *sarson*, Chinese cabbage, *Brassica campestris* L. var. *pekinensis* and *taramira*, *Eruca sativa* Lam. var. ITSA differed qualitatively as well as quantitatively mainly with respect to allyl-, methylthiobutyl-, pent-4-enyl-, methyl- and benzyl-glucosinolates. The mean number of total probes on feeding media incorporated with crude extracts containing glucosinolates from 0·25, 0·50 and 1·0 g green parts of *taramira* per ml of feeding media was 5·8, 6·7 and 8·4, respectively. Such probes in case of yellow *sarson* and Chinese cabbage were 2·6, 2·2 and 1·5; and 2·6, 2·0 and 3·0, respectively. The mean number of probes on feeding media containing sinigrin (K^+-allylglucosinolate) at the rate of 0·125, 0·25, 0·50 and 1·0% was 1·9, 3·3, 3·9 and 5·2, respectively. Uptake of feeding media incorporated with extracts containing glucosinolates from *taramira*, yellow *sarson* and Chinese cabbage varied from 4·05–9·40, 31·5–52·0 and 30·5–51·3%, respectively. Similarly, the uptake in the case of feeding media containing sinigrin (0·125–1%) varied from 1·6–44·95%.

Keywords. Glucosinolates; sinigrin; probing pattern; feed uptake; Crucifers; *Lipaphis erysimi* (Kaltenbach).

1. Introduction

Cruciferous plants are attacked by a number of insect species. Host range of these insect species is restricted to a few closely related plant families which contain glucosinolates. These compounds act as semiochemicals against these specialist feeders (Verschaeffelt 1911; Thorsteinson 1953; Hovanitz 1969; Feeny *et al* 1970; Dethier 1972; van Emden 1972; Cole and Finch 1978). One of these compounds, allylglucosinolate affected feeding among aphids, *Brevicoryne brassicae* (Wensler 1962; Moon 1967; van Emden 1972), *Hyadaphis* (=*Lipaphis*) *erysimi* (Nault and Styer 1972; Narang 1982) and *Myzus persicae* (van Emden 1972) by affecting their probing behaviour.

This paper further reports on the effects of glucosinolates on the probing behaviour and feed uptake by mustard aphid, *Lipaphis erysimi* (Kalt.). These investigations were carried out as a part to the studies on the host selection mechanism of this aphid.

2. Materials and methods

Two of the 3 crucifers selected for the present studies, yellow *sarson*, *Brassica campestris* L. var. yellow *sarson*; Chinese cabbage, *B. campestris* L. var. *pekinensis* are susceptible and third, *taramira*, *Eruca sativa* Lam. var. ITSA, is resistant to the attack of mustard aphid, *Lipaphis erysimi* (Kalt.). For the purpose of extraction and analysis

of glucosinolates, 35 day old plant samples were taken from infestation free field sown crops. The plants were uprooted, washed and dried with a blotter. The roots were clipped off and rest of the plant material was quickly stored in a deep-freeze for further analysis.

2.1 *Analysis of glucosinolates*

Intact glucosinolates were extracted from 100 g freeze-dried vegetative parts of yellow *sarson*, Chinese cabbage and *taramira* as described by Olsen and Sorensen (1980). Concentrated crude extract equivalent to 25 g plant material (Ca 2·5 ml) was adsorbed on a column containing activated charcoal (2·5 g) and cellulose (5 g) as bed. The glucosinolates were eluted with 100 ml of 70% ethanol. The eluted material was free from pigments. It was concentrated to 5 ml, out of which 1 ml concentrate was kept separately for bioassay studies and the remaining 4 ml was further analysed for glucosinolate content by following the method of Olsen and Sorensen (1980) with certain modifications.

Two glass columns containing Amberlite IR-120$^+$ (1·8 × 50 cm) and Ecteolla cellulose ACo$^-$ (1·8 × 60 cm) were serially connected. Crude extract of glucosinolate (4 ml) equivalent to 20 g plant material was transferred to Amberlite column. After washing columns with 500 ml distilled-decarbonised water, the glucosinolates were eluted from ACo$^-$ column with 500 ml 1 M pyridine. The eluted material was evaporated to dryness and redissolved in 5 ml 80% ethanol. The glucosinolate mixtures were separated following two dimensional chromatography on silica gel-G (E Merck, Germany) 0·20 mm, preactivated plates using n-butanol : n-propanol : acetic acid : water : 3 : 1 : 1 : 1 (Underhill and Kirkland 1971) as first system and n-butanol : benzene : ethanol : ammonia : 4 : 1 : 1 : 2 as the second system. Similarly, derivatised sinigrin was used as standard. The spots were visualized by spraying alkaline silver nitrate. The R_f values were expressed with respect to allylglucosinolate. Spots were identified as per Rakow *et al* (1981). The relative concentrations were estimated by comparing area of spot against amount of plant sample and standard sinigrin sample (Aldrich Chem. Co., USA).

2.2 *Probing behaviour*

Probing behaviour was studied on parafilm-M membrane sachets containing a basal medium of 30% sucrose + 6 amino acids (alanine 60, aspargine 180, glutamine 360, leucine 120, methionine 60 and valine 120 mg per 100 ml) and a known amount of crude extract of plants containing glucosinolates. Sinigrin (K$^+$-allylglucosinolate) was used as a test. The basal medium containing extracts of plant material or sinigrin would now be called a feeding medium.

Parafilm sachets were prepared as described by Mittler and Dadd (1964). Each sachet was provided with 0·2 ml of feeding medium and fixed over a glass annulus (2 × 2·1 cm). One aphid, 3–4 instar, was released at a time from the open end of glass annulus which rested over the stage of stereoscopic zoom binocular. The aphid was attracted towards the feeding site by placing a small piece (3–4 mm) of yellow cellophane paper. The aphid was viewed through the binocular continuously. With the help of a stopwatch, probing period was recorded. Since a large number of aphids

had to be observed, probings lasting longer than 5 min were discontinued. On the basis of data available on all the feeding media, the frequency of probing duration was distributed into following 5 classes: 0–15, 16–30, 31–60 s, 1 to less than 5 min, and 5 min and above. The probe made by an aphid for 1 min or more was termed as feeding probe. An aphid which had made a feeding probe was discarded. All feeding media were evaluated with 10 aphids each.

2.3 *Feed uptake*

The intake of feeding medium by this insect was evaluated with the help of radioisotope technique. The source of radioactivity was sucrose-C^{14} (U) with a specific activity of 480 mCi/m mole. Labelled sucrose was incorporated in all the feeding media.

Feeding media containing sucrose-C^{14} and giving count rate of 45000–50000 per min were offered in parafilm membrane sachet to 10 aphids of 2nd-3rd instar to feed upon for 48 h. After this, the sachet was cut with pointed razor blade and immersed in 5 ml of scintillation fluid in scintillation vials. The tightly capped vials were shaken vigorously to release the total contents of the sachet into the scintillation fluid. Three sets of sachets were prepared. Two sachets were offered to insects for feeding, while the third one was kept as control to work out the radioactivity taken up by the aphids. The leftover radioactivity was measured. The radioactivity was measured by Liquid Scintillation Spectrometer (Packard, Model LC 100) using scintillation fluid as described by Bray (1960). The per cent radioactivity taken up would be proportional to the amount of the feeding media taken up by the aphids.

3. Results

3.1 *Plant analysis*

The composition and concentration of glucosinolates during the vegetative stage of yellow *sarson*, Chinese cabbage and *taramira* are given in table 1. Methylthiobutyl-, methyl- and benzylglucosinolates were detected in *taramira* only and the relative concentration of the former was estimated to be higher than that of the latter two. Hydroxypent-4-enyl- and pent-4-enylglucosinolates were detected in yellow *sarson* and Chinese cabbage. Allyl- indo-3-ylmethyl- and phenylethyl-glucosinolates were estimated in higher amounts in *taramira* than that in other two hosts, whereas the reverse was true for but-3-enylglucosinolate. Yellow *sarson* contained higher amounts of hydroxy but-3-enylglucosinolate, whereas it was methyl-sulphinylpentyl in the case of Chinese cabbage. Hydroxybenzyl-glucosinolate was detected in yellow *sarson* and *taramira* only.

3.2 *Effect of glucosinolates on the probing pattern*

The probing pattern of mustard aphid on various feeding media is given in figure 1. However, the mean number of total probes on feeding media incorporated with extracts containing glucosinolates from 0·25, 0·50 and 1 g green parts of *taramira* per

Table 1. Composition of glucosinolates in test cultivars.

Name of glucosinolate	Relative concentration: Yellow *sarson*	Chinese cabbage	Taramira	RA[b]
Methylglucosinolate	–	–	+	0·76
Methylsulphinylpentyglucosinolate	+ +	+ + + +	+ +	0·92
Allylglucosinolate	+	+	+ + + +	1·00[a]
Ho-but-3-enylglucosinolate	+ + +	+ +	+ +	1·00[a]
Ho-pent-4-enylglucosinolate	+ +	+ +	–	1·00[a]
Ho-benzylglucosinolate	+	–	+ +	1·10[a]
Methylthiobutylglucosinolate	–	–	+ + + +	1·25
But-3-enylglucosinolate	+ + + +	+ + + +	+ +	1·50[a]
Benzylglucosinolate	–	–	+	1·54
Indo-3-ylmethylglucosinolate	+ +	+ +	+ + +	1·55
Pent-4-enylglucosinolate	+ +	+ + +	–	1·62[a]
Phenylethylglucosinolate	+ +	+ +	+ + +	1·75[a]

[a]Confirmed identifications as per Rakow *et al* (1981). Rest of the identifications are extrapolated and tentative.

[b]R_f values are relative to allylglucosinolate.

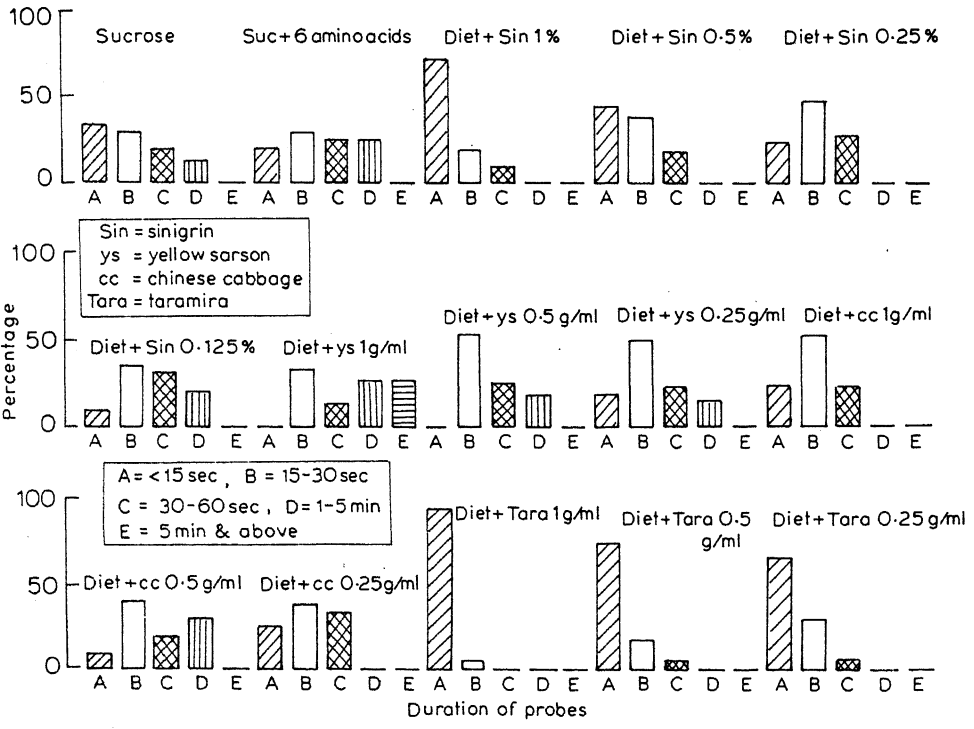

Figure 1. Relative frequency distribution of duration of probes made by *L. erysimi* (3–4 instar) into various feeding media via parafilm membrane.

ml of feeding media was 5·8, 6·7 and 8·4, respectively. Such probes in the case of yellow *sarson* and Chinese cabbage were 2·6, 2·2, 1·5 and 2·6, 2·0, 3·0, respectively. The mean number of total probes in the case of 0·125%, 0·25%, 0·50% and 1% sinigrin (K^+-allylglucosinolate) containing feeding media was 1·9, 3·3, 3·9 and 5·2, respectively (table 2). The mean number of total probes was significantly higher in the case of feeding media containing glucosinolate extracts of *taramira* and higher concentration of sinigrin (1%) than those containing glucosinolate extracts of yellow *sarson*, Chinese cabbage and the lower concentration of sinigrin (0·125%).

The proportion of feeding probes was higher in the case of basal medium (diet) alone, media containing sinigrin (0·125%), and glucosinolate extracts of yellow *sarson* (0·25–1 g/ml) and Chinese cabbage (0·25–0·5 g/ml) as compared to that in the other treatments (figure 2). In the case of *taramira* and sinigrin (1%), the proportion of feeding probes was almost negligible.

3.3 *Effect of glucosinolates on feed uptake*

Uptake of feeding media incorporated with extracts containing glucosinolates from 0·25, 0·50 and 1 g green parts of yellow *sarson* per ml of feeding media was 40, 31·5 and 52%, respectively. The feed uptake was significantly higher at 1 g level than that in the case of other two. In the case of feeding media incorporated with extracts containing glucosinolates from 0·25, 0·50 and 1 g of Chinese cabbage per ml of feeding media, the uptake was 44·4, 51·30 and 30·25%, respectively. The uptake at 0·5 g level was significantly higher than that in the case of other two. The uptake in the

Table 2. Probing behaviour of mustard aphid, *L. erysimi* (Kalt.) on different feeding media.

	Mean number of probes and proportion of feeding probes per 5 min	
Feeding medium	Mean	Proportion of feeding probes
Sucrose	$2·9^{abcd*}$	0·34
Sucrose + 6 a.a. (diet)	$2·4^{abc}$	0·50
Diet + Sinigrin 1%	$5·2^{e}$	0·08
Diet + Sin 0·5%	$3·9^{cd}$	0·18
Diet + Sin 0·25%	$3·3^{bcd}$	0·27
Diet + Sin 0·125%	$1·9^{ab}$	0·53
Diet + Yellow *sarson* 1 g/ml	$1·5^{a}$	0·67
Diet + Ys 0·5 g/ml	$2·2^{abc}$	0·45
Diet + Ys 0·25 g/ml	$2·6^{abc}$	0·31
Diet + Chinese cabbage 1 g/ml	$3·0^{bcd}$	0·23
Diet + Cc 0·5 g/ml	$2·0^{ab}$	0·50
Diet + Cc 0·25 g/ml	$2·6^{abc}$	0·35
Diet + Taramira 1 g/ml	$8·4^{g}$	0·01
Diet + Tara 0·5 g/ml	$6·7^{f}$	0·06
Diet + Tara 0·25 g/ml	$5·8^{ef}$	0·05
SE (m)	0·440	

*Figures followed by a common letter in the column do not differ significantly at $P = 0·05$ according to Duncan's multiple range test.

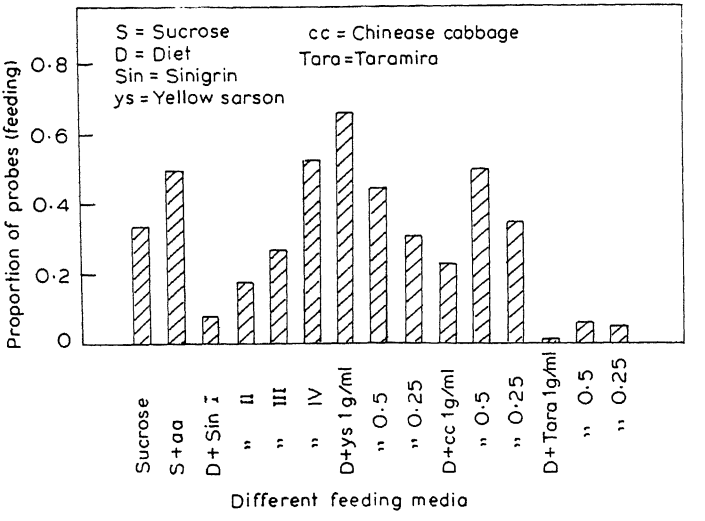

Figure 2. Proportion of probes made by *L. erysimi* as feeding probes on various feeding media.

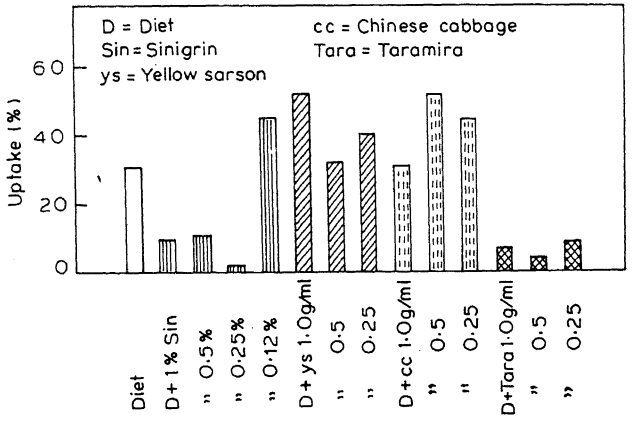

Figure 3. Per cent uptake of radioactivity proportional to feed uptake.

case of *taramira* (0·25–1 g) was very low and varied from 9·4–4·05%, the difference being non-significant. Similarly, in case of sinigrin (0·25–1%), the uptake was very low but at 0·125% level, it was 44·95% which was significantly higher than that in all the other levels (figure 3).

The uptake in the case of yellow *sarson* (1 g), Chinese cabbage (0·25 and 0·50 g) and sinigrin (0·125%) was of a similar magnitude and was significantly higher than that of *taramira* (0·25–1 g) and sinigrin (0·25–1%) (table 3).

At the end of observation period of 48 h, it was found that most of the aphids feeding on the media containing glucosinolate extracts of *taramira*, and 0·25–1% sinigrin were dead. Even in the case of feeding medium containing 0·125% sinigrin,

Table 3. Response of 2–3 instar nymphs of *L. erysimi* (Kalt.) to various feeding media through parafilm membrane.

Treatment	Uptake of feeding media (%)			Aphids surviving after 48 h (%)
	Ist set	2nd set	Average	
Diet + Sinigrin				
1%	9·5	9·9	9·7	0
0·5%	2·7	18·7	10·7	10
0·25%	2·6	0·6	1·6	0
0·125%	52·4	37·5	44·95	60
Diet + Taramira				
1 g/ml	8·5	7·0	7·75	0
0·5 g/ml	0·4	7·7	4·05	0
0·25 g/ml	0·0	18·8	9·4	10
Diet + Yellow *sarson*				
1 g/ml	58·4	45·6	52·0	80
0·5 g/ml	27·8	35·2	31·5	100
0·25 g/ml	49·7	30·3	40·0	100
Diet + Chinese cabbage				
1 g/ml	27·7	32·8	30·25	100
0·5 g/ml	49·3	58·3	51·30	100
0·25 g/ml	50·2	38·6	44·4	100
Standard diet	39·4	23·7	31·55	
SE			4·96	
Value of '*t*'			5·32*	

*Significant at 5%.

40% of the aphids died. It showed that although at this level sinigrin stimulated stylet penetration yet it was not favourable for the survival.

The aphids which were surviving on feeding media containing glucosinolate extracts of *taramira* (0·25 g) were found to be in poor health and wingpads had started appearing. In all the other treatments having glucosinolate extracts of yellow *sarson* and Chinese cabbage and basal medium alone, the aphids survived well and were in good health.

A significant correlation existed between the uptake of feeding media and mean number of probes per 5 min (−0·786*) and in between uptake and proportion of feeding probes (0·848*). The coefficient of correlation between mean number of probes and proportion of feeding probes (−0·898*) was also significant. These relationships indicate that the duration of probes was proportional to feed uptake and the criterion selected to decide feeding probe was valid. It showed, if an aphid probes for 1 min or more on a parafilm membrane sachet, it would result in feed uptake.

4. Discussion

Artificial feeding techniques are of immense importance in evaluating the chemicals associated with host-plants. It is of particular importance for aphids which are

normally host-specific. The use of parafilm membrane by Bradley (1956) in studying the mechanism of virus transmission by aphids was followed by considerable amount of work on aphid nutrition (Auclair 1976).

Parafilm membrane was used by Moon (1967), Wearing (1968) and Nault and Styer (1972) for studying the role of allylglucosinolate on host-selection of *B. brassicae*, *M. persicae* and *Hyadaphis erysimi*. Behavioural components, such as durations of probe, first walk or first probe, and frequency of probes, have been studied by different workers to explain the host-selection mechanism.

In the present investigations, duration and frequency of probes, proportion of feeding probes and uptake of feeding media as affected by cruciferous glucosinolates were studied. Differences in the probing behaviour and feed uptake could be attributed to the qualitative and quantitative differences in glucosinolate contents of the 3 hosts (table 1). A compound appearing to be allylglucosinolate was present in the 3 hosts, but its concentration was higher in *taramira* than that in the other two hosts. The semiochemical nature of allylglucosinolate has been reported with respect to a number of insects feeding on crucifers including aphids (Wensler 1962; Moon 1967; van Emden 1972; Nault and Styer 1972). Therefore, it was used as the test compound in the present studies.

Probing behaviour and feed uptake in the case of feeding media containing extracts from *taramira* were similar to those feeding media which contained higher concentrations of sinigrin (0·25–1%). Similarly, these parameters in the case of feeding media containing extracts from yellow *sarson* and Chinese cabbage were similar to those which had the lowest concentration of sinigrin (0·125%).

Extracts of green parts of *taramira*, which were richer in allylglucosinolate content than those of yellow *sarson* and Chinese cabbage (table 1), probably deterred the feeding of *L. erysimi* by affecting the probing pattern. The dose dependent response to sinigrin indicated that variation in its concentration in the 3 hosts alone could trigger such a response in mustard aphid.

The possibility of involvement of other glucosinolates of the host plants for eliciting differential responses of mustard aphid cannot, however, be ruled out. This could not be tested experimentally owing to the non-availability of other standard compounds.

There is no evidence available in literature to support the present findings, except that the allylglucosinolate stimulated stylet penetration of *Hyadaphis* (=*Lipaphis*) *erysimi* (Nault and Styer 1972). However, it was also found to influence the stylet penetration of *B. brassicae* on treated substrates (Wearing 1968; van Emden 1972). According to Nault and Styer (1972), even non-host plants attracted aphids of *B. brassicae* and *H. erysimi* in large number when treated with 1% allylglucosinolate Both of these aphid species are specialist feeders on crucifers. However, a polyphagous species, *M. persicae* did not show improvement in its feeding when allylglucosinolate was provided in the feeding medium (van Emden 1972; Schoonhoven and Derksen-koppers 1976).

There was a direct relationship between proportion of feeding probes and feed uptake—a phenomenon also observed earlier by van Emden (1972). It is expected that lower feed uptake might have influenced adversely the survival, development and multiplication of aphids on *taramira*. Narang (1982) had also observed poor survival, development and multiplication of this aphid on *taramira*. The possibility of antibiotic mechanism after ingestion appeared to be of secondary importance as the

presence of allylglucosinolate in higher amounts in *taramira* deterred the feed uptake by affecting the process of probing on the host plant.

It appears, therefore, that cruciferous glucosinolates influence frequency and duration of probing which would ultimately decide the feed uptake. Such an allelochemic interaction between cruciferous glucosinolates and the mustard aphid might be playing a role in the latter's host selection mechanism.

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Energy utilization in freshwater mullet, *Rhinomugil corsula* (Hamilton) under exercise

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Abstract. Energy utilization in mullet, *Rhinomugil corsula*, exercised continuously for 5 h at different swimming speeds ranging between 20 and 77 cm s^{-1} was studied in a tunnel type apparatus. Oxygen consumption, carbon dioxide output and respiratory quotient decreased with increase in the duration of exercise. The initial (1st h) respiratory quotient, which was always above unity, increased with intensity of swimming speed, but the steady (5th h) respiratory quotient remained below unity, at about the same level irrespective of the increase in activity.

Nitrogen (NH_3 and total) excretion, ammonia quotient and nitrogen quotient increased with both duration and intensity of exercise, indicating the increased protein utilization during the later phase of exercise.

An attempt has been made to estimate the relative energy derivation from different substrates with some assumptions for the entire 5 h of exercise at 20 and 77 cm s^{-1}. At 20 cm s^{-1} the energy derived aerobically from proteins, carbohydrates and fats and that from anaerobic source (carbohydrate) were estimated as 13040·3, 1378·2, 2367·3 and 1048·5 J kg^{-1} fish, being 73·1, 7·7, 13·3 and 5·9% of a total of 17834·3 J kg^{-1} fish, whereas at a swimming speed of 77 cm s^{-1} it was estimated as 33587·9, 2841·4 and 1305·4 J kg^{-1} fish from proteins, fats and anaerobic source respectively, being 89·0, 7·5, 3·5% of a total of 37734·7 J kg^{-1} fish.

Keywords. Energy utilization; *Rhinomugil corsula*; oxygen consumption; respiratory quotient; ammonia quotient; nitrogen quotient.

1. Introduction

Considerable information has been accumulated on the physiology of fish subjected to exercise (forced activity) as reviewed by Fry (1971), Brett (1979) and Beamish (1979). Much of this pertains to the study of respiratory metabolism (Brett 1964, 1973; Smit *et al* 1971; Kutty 1968; Rao 1968; Kutty and Peer Mohamed 1975; Sukumaran and Kutty 1977; Sukumaran 1986; Jones and Randall 1979; Paulson 1980) and also tissue metabolism especially with reference to changes in substrates and end products of metabolism in muscle and blood (Black 1958; Black *et al* 1962; Karuppannan 1972; Johnston and Goldspink 1973; Johnston 1975; Woleoma and Johnston 1981; Jones 1982). In general it is noticed that while there are several studies on respiratory metabolism and some aspects of substrate utilization and end product accumulation and disposal, there is no study which has been specifically done combining the physiological parameters under same set of experimental conditions so as to have a comprehensive information on the total metabolism of fish especially with reference to energy utilization connected with swimming activity of fish. The objective of present study is to obtain a comprehensive picture of energy utilization in swimming mullet from two levels, one depicting the total changes in the

intact fish, from the measurement of oxygen consumption and excretion of carbon dioxide and nitrogen and the other depicting the biochemical changes in muscle, liver and blood of fish subjected to different level and periods of exercise. An attempt is also made to estimate the total energy utilization of the swimming fish.

2. Material and methods

The freshwater mullet, *Rhinomugil corsula*, used for this study were collected from Vaigai Reservoir, near Madurai and held in holding tanks in the laboratory. They were acclimated to freshwater at $30 \pm 1°C$ for atleast two weeks before experiment and tested under acclimation conditions. They were fed once daily with a formulated diet, prepared out of rice bran and groundnut oil cake in the ratio of 2:1 (Narayanan 1974). The fish were starved for 24 h (Beamish 1964) before experiment.

The experiments were performed in Blazka's respirometer (Blazka *et al* 1960) described by Sukumaran and Kutty (1977). In the apparatus the fish was made to swim at different swimming speeds (20, 38, 62 or 77 cm s^{-1}) continuously for 5 h. The metabolic rates were measured by closing off the flow of water through the respirometer for 1 h, during 1st, 3rd and 5th h (alternate hours of continuous 5 h exercise). Water samples were taken during the beginning and end of closure period. All such closure periods were followed by an hour's flushing with air saturated water.

For analysis of the muscle (red and white) and liver glycogen and blood glucose and lactate, the exercise was terminated at 1st, 3rd and 5th h; the fish was removed immediately from respirometer and was killed. Blood samples were taken in a haparinized syringe from direct heart puncture and transferred to a vial, under liquid paraffin. The tissues were separated immediately and kept in deep freeze. The removal and fixing of tissue for analysis took 5 min.

The dissolved oxygen in water was analysed following unmodified Winkler method (APHA 1965). In some cases the dissolved oxygen was determined by a YSI (Yellow Spring Institute) oxygen electrode. Total carbon dioxide in water was analysed within an hour after sampling following the Maros-Schulek technique as modified by Kutty *et al* (1971). Fifty ml of samples were used for analysis. The samples were analysed for ammonia following the method described by Kutty (1972), which is in agreement with Strognov (1962) and APHA (1965). The total nitrogen of water samples were analysed following the method described by Jacobs (1951), using a steam distillation micro-kjeldahl apparatus.

The lactic acid in muscle tissue (white and red), liver and blood was estimated, following the method of Barker and Summerson (1941) as outlined by Oser (1965). The glycogen in muscle and liver tissue was precipitated according to the method of Seifter *et al* (1949) and the precipitated glycogen was assayed by the anthrone method of Carroll *et al* (1956). Blood glucose was analysed following the anthrone method (Carroll *et al* 1956).

3. Results

Oxygen consumption, Co_2 output and respiratory quotient (RQ)* measurements obtained are plotted against swimming speed in figure 1. Regression lines have been

*The mol to mol ratio of Co_2 excreted to O_2 consumed also known as respiratory exchange ratio.

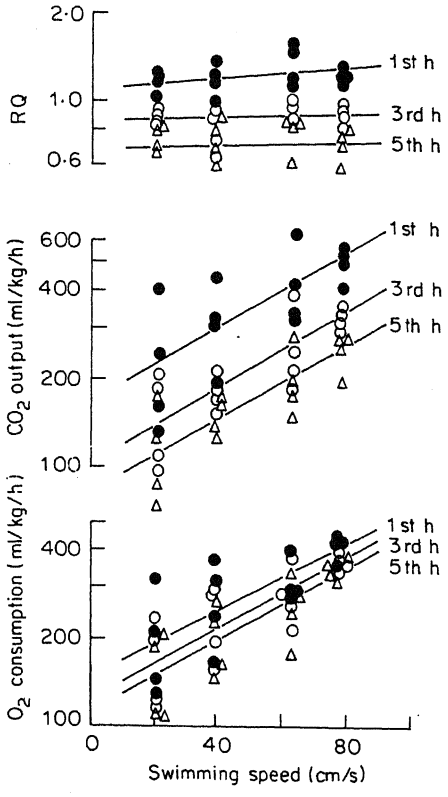

Figure 1. O_2 consumption, CO_2 output and RQ in relation to swimming speed in *R. corsula*, forced to swim continuously for 5 h in freshwater at 30°C. The regression lines fitted are as per equations presented in table 1.

fitted separately for each hour of exercises, as per the equations given in table 1. The rate of ammonia-N and total-N excretion and ammonia quotient (AQ) and nitrogen quotient (NQ) are calculated from the available data and depicted in semilogarithmic grid against swimming speed in figures 2 and 3 respectively. The plots are fitted with regression lines as per the equations given in table 1. The glycogen utilization and lactate production during 5 h exercise at 20 and 77 cm/s are presented in table 2.

It is clear from figures 1–3 that the metabolic rates increased with the increase of swimming speed, whereas the metabolic rate during the initial phase of exercise (1st h) was found to be higher (figure 1) than the rate of oxygen consumption during the later phase of exercise (5th h). It is also evident from figure 1 that the 1st h RQ values were slightly above unity, suggesting the involvement of anaerobic metabolism during initial phase of exercise. The rate of nitrogen excretion as well as the respective quotients increased not only with the increase of swimming speed but also with increase in the duration of exercise (figures 2 and 3) indicating the higher protein utilization during later phase of exercise in all the speeds tested.

4. Discussion

The oxygen consumption in *R. corsula* increases with increase of swimming speed

Table 1. Regression equations of logarithms of oxygen consumption, carbon dioxide output (both in ml/kg/h) and nitrogen (ammonia-N and total-N) excretion (mg/kg/h) RQ, AQ and NQ (Y in each case) against swimming speed (cm/s) (X).

	Exercise (h)	Regression equation $\log Y = a + bx$
O_2	1	2·17415 (±0·12114) + 0·005763 (±0·001392) X
	3	2·09825 (±0·10310) + 0·006137 (±0·001204) X
	5	2·04735 (±0·10380) + 0·006302 (±0·001124) X
CO_2	1	2·22213 (±0·12479) + 0·006815 (±0·001194) X
	3	2·01617 (±0·09486) + 0·006895 (±0·001936) X
	5	1·92653 (±0·15710) + 0·006510 (±0·002828) X
NH_3-N	1	1·03087 (±0·02092) + 0·008037 (±0·000114) X
	3	1·19430 (±0·02937) + 0·008114 (±0·000107) X
	5	1·29068 (±0·05618) + 0·008172 (±0·000562) X
Total-N	1	1·19875 (±0·081701) + 0·007763 (±0·001105) X
	3	1·34655 (±0·05656) + 0·008030 (±0·000387) X
	5	1·46723 (±0·07566) + 0·007980 (±0·000659) X
RQ	1	0·04588 (±0·01830) + 0·001037 (±0·000387) X
	3	−0·06687 (±0·02291) + 0·000569 (±0·000296) X
	5	−0·12671 (±0·02828) + 0·000278 (±0·000278) X
AQ	1	−1·29487 (±0·11467) + 0·002201 (±0·001479) X
	3	−1·05779 (±0·06245) + 0·001688 (±0·000696) X
	5	−0·91124 (±0·11576) + 0·001862 (±0·001432) X
NQ	1	−1·13023 (±0·10712) + 0·001964 (±0·001673) X
	3	−0·88739 (±0·05518) + 0·001345 (±0·000823) X
	5	−0·73618 (±0·04336) + 0·001686 (±0·000721) X

Data obtained for 1st, 3rd and 5th h of 5 h continuous exercise at 30°C. Values in parentheses indicate one SE (N = 16 in each case).

and decreases with duration of exercise (figure 1) as obtained previously for Sockeye salmon (Brett 1964) gold fish (Smit 1965; Kutty 1968; Rao 1968) and *Tilapia mossambica* (Karuppannan 1972). The carbon dioxide output in *R. corsula* also showed the same trends as observed earlier in gold fish and rainbow trout (Kutty 1968) and in *T. mossambica* (Karuppannan 1972). The initial RQ values are always higher than unity, whereas the steady (long-term) RQ values are below unity, suggesting that anaerobic energy utilization is high during the initial phase of swimming exercise, but not during later phase as observed in other fishes.

Nitrogen (ammonia and total) excretion increases sharply with increase in the duration of exercise and with the intensity of swimming speed (figures 2 and 3). In previous studies the exercised fish (Kutty 1972; Karuppannan 1972), NH_3-N excretion has only been considered as a measure of protein degraded. In the present study a better picture is obtained by measuring also the total-N excretion. The percentage of NH_3-N in total nitrogen excreted varies very little in duration of exercise as well as the intensity of exercise (65·6–67·3% for speed increased of 20–77 cm/s and decreased from 67·3–65·5% for the 1st to 5th h of exercise at the highest speed tested). It however, appears that the excretion of non-ammonia fraction is also increasing with the duration of exercise and intensity of exercise, while the proportion of it to total nitrogen excretion is always staying constant.

The quotients of NH_3-N excretion and total N excretion show the same trend, to

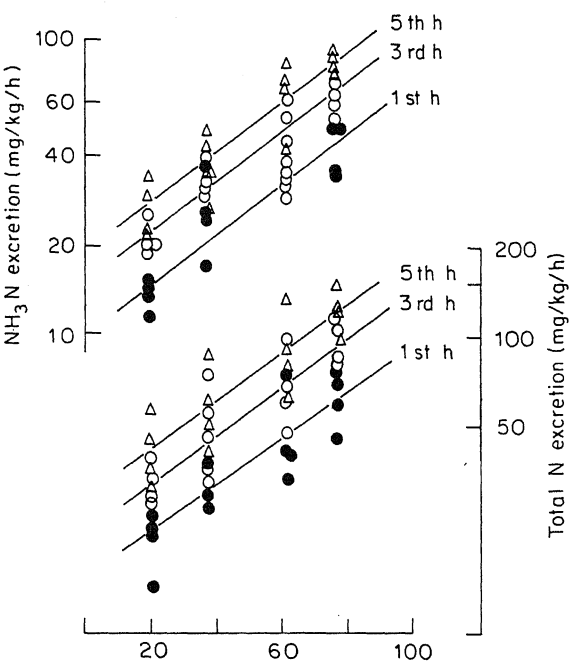

Figure 2. AN and total nitrogen excretion in relation to swimming speed in *R. corsula* forced to swim continuously for 5 h in freshwater at 30°C. The regression lines fitted are as per the equations given in table 1.

increase with increase in intensity and duration of exercise. In the present study the AQ and NQ are the weight relations of N-excreted and oxygen consumed, unlike the AQ of earlier workers (Kutty 1972; Karuppannan 1972). The values of AQ and NQ are relatively lower during short-term exercise (1 h), whereas the long-term values are higher, as observed again in the case of *Tilapia* AQ by Kutty (1972), Karuppannan (1972) and Sukumaran (1986), suggesting that relative protein utilization is lower during initial phase of exercise than in later phase in all the swimming speeds tested. It was suggested that this may be due to the sparing action of carbohydrates on proteins (Kutty 1981).

The glycogen concentration of both white and red muscles and liver decreases after 5 h exercise (at 20 and 77 cm/s) in mullet, whereas the lactate concentration increases (table 2). The blood glucose rises during the initial phase of exercise and the level is maintained with a slight decrease during the later phase of exercise. The total glycogen utilization of *R. corsula* at various hours of exercise has been estimated considering the changes in muscle (white and red), liver glycogen and blood glucose (Johnston and Goldspink 1973; Woleoma and Johnston 1981). In our calculations the glycogen changes are always expressed in equivalent amount of glucose. Data obtained for glycogen utilization and lactate production during the 1st h of exercise in mullet, exercised at 20 cm/s can be considered as a typical example for analysis.

Estimation of glycogen utilization for the 1st h of swimming at 20 cm/s in *R. corsula*:

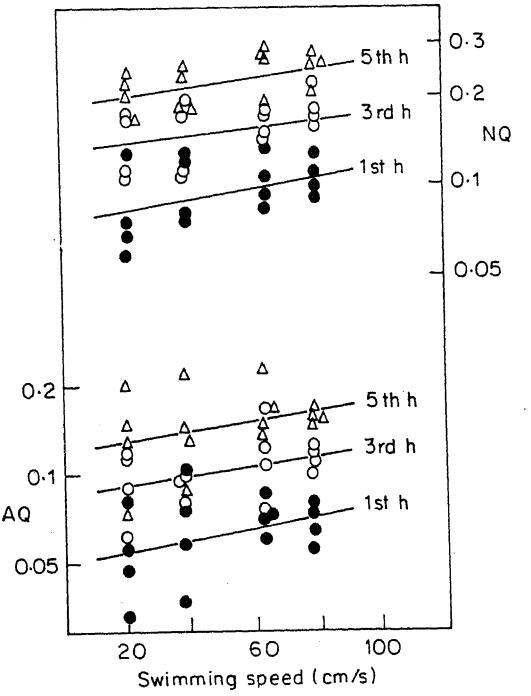

Figure 3. AQ and NQ in relation to swimming speed in *R. corsula*, forced to swim continuously for 5 h in freshwater at 30°C. The regression lines fitted are as per the equations given in table 1.

Table 2. Glycogen (glucose) utilization in m mol/kg (fish)$^{-1}$ of *R. corsula* exercised continuously for 5 h at 20 and 77 cm s^{-1}.

Swimming speed cm/s	Duration of exercise (h)	Glycogen (glucose) utilized for lactate production	Glycogen (glucose) utilized for maximum oxidation	Unaccounted glycogen (glucose)	Total glycogen (glucose) utilized by the fish
20	1	2·06 (30·3)	1·50 (22·1)	3·22 (47·5)	6·78 (99·9)
20	5	4·82 (31·0)	6·46 (41·5)	4·28 (27·5)	15·56 (100)
77	1	3·18 (21·6)	3·10 (21·1)	8·43 (57·3)	14·71 (100)
77	5	6·01 (33·7)	11·78 (66·2)	Nil	17·79 (99·9)

Percentage of total glycogen utilized are indicated in parentheses.

Total glucose utilized = 6·78 m mol kg^{-1} h^{-1}.
Total lactate produced = 4·12 m mol kg^{-1} h^{-1}.
Total O_2 consumed in 1st h = 285·6 mg kg^{-1} h^{-1}.

Glucose needed for the production of 4·12 m mol of lactate $= \frac{4·12}{2} = 2·06$ m mol kg^{-1}h^{-1}.

Assuming that all the O_2 consumed by the fish is utilized for glycogen (glucose) oxidation.

$$\text{Glucose (possible maximum) oxidised} = \frac{285{\cdot}6}{192} = 1{\cdot}50 \text{ m mol kg}^{-1} \text{ h}^{-1}.$$

Total glucose utilized for lactate production and maximum possible oxidation $= 2{\cdot}06 \times 1{\cdot}50 = 3{\cdot}56$ m mol kg^{-1} h^{-1} unaccounted glycogen (glucose) $= 6{\cdot}68 - 3{\cdot}56 = 3{\cdot}12$ m mol kg^{-1} h^{-1}.

Likewise the breakups for glycogen (glucose) utilization on mullet for the 1st h of 77 cm/s swimming speed and the entire 5 h exercise at both low and high speeds (20 and 77 cm s^{-1}).

It is clear that about half of total glycogen utilized during the 1st h of exercise has been unaccounted. While the unaccounted percentage of glycogen is low in the entire 5 h period of swimming at 20 cm/s and it is nil at 77 cm/s. It should be mentioned here that the entire oxygen consumed by the fish is assumed to be utilized only to oxidize glucose. But it is certain that other substrates are also involved in the oxidative metabolism, since a major part of energy specially at later hours of exercise is derived from protein oxidation, as obvious from the increase in nitrogen excretion during the later hours of exercise (figures 2 and 3). The unaccounted glucose in all the levels might be utilized for the production of end products other than lactate. Hochachka (1980) observed the accumulation of pyruvate and alanine as the end products of glycolysis in carp white muscle. Hochachka *et al* (1974) found that several other end products (such as pyruvate, succinate and alanine) accumulated in diving vertebrates. It is possible that in fish also such anaerobic and products are accumulating. Kutty and Peer Mohamed (1975) have suggested that in mullet, *R. corsula* anaerobic energy is derived under hypoxia through pathways other than conventional glycolysis. The unaccounted glycogen (glucose) in exercised mullet might have also been used for deriving energy efficiently through some anaerobic pathway other than conventional glycolysis.

Another possibility, though less likely is that the unaccounted glycogen (glucose) can be converted into other substrates namely fat and some amino acids. Nagai and Ikeda (1971) and Blazka (1958) suggested that in crucian carp under ammonia glycogen is converted to fat, resulting in the release of CO_2 and extra energy. Nagai and Ikeda (1971) studying the carbohydrate metabolism in carp, showed the incorporation of glycose C^{14} mostly in amino acids (protein) and some in lipids. This clearly suggests the conversion of glucose into other energy substrates namely fat and proteins. But in exercised mullet how significant such a conversion is cannot be known from available data.

The present study again facilitates some estimates of relative energy utilization under certain assumptions. The method followed is similar to that described by Kutty and Peer Mohamed (1975), Kutty (1972, 1981) and Sukumaran (1976) estimated that a maximum AQ (molar ratio of NH_3 excreted to O_2 consumed) 0·33 can be expected for breakdown of protein under fully aerobic constitution. The compiling value of weight of nitrogen excreted to O_2 consumed i.e. maximum aerobic NQ is 0·148. In our calculation, shown under it is assumed that an observed NQ above that value indicates the excretion of anaerobic nitrogen. Thus aerobic nitrogen excretion is estimated by substracting the anaerobic from the total nitrogen excretion. With these assumptions, the energy utilization of *R. corsula* exercised at

20 cm/s, for the 5 h period of swimming is tabulated below:

Total O_2 consumed for 5 h	= 868·1 ml kg^{-1} or = 1240·1 mg kg^{-1}.
Total CO_2 output for 5 h	= 832·9 ml kg^{-1}.
Total N excreted for 5 h	= 161·6 mg kg^{-1}.
Estimated anaerobic N-excretion	= 17·4 mg kg^{-1}.
Therefore, total aerobic N excreted	= 161·6 − 17·4 = 144·2 mg kg^{-1}.
O_2 needed to degrade proteins to produce 144·2 mg kg^{-1} of nitrogen	= 144·2/0·148 = 974·3 mg kg^{-1}. = 682·0 ml kg^{-1}.
CO_2 equivalent of 682·0 ml kg^{-1} of O_2, in protein breakdown	= 682·0 ml kg^{-1}.
[Since the protein RQ is calculated to be unity for fish system (Kutty 1981; Sukumaran 1976)].	
Non protein (NP) O_2	= 868·1 − 682·0 = 186·1 ml kg^{-1}.
NP CO_2	= 832·9 − 682·0 = 150·9 ml kg^{-1}.
NP RQ	= 150·0/186·1 = 0·81.

Since the non-protein RQ is clearly below unity, it is possible to estimate the proportionate energy derivations from the 3 substrates, proteins, carbohydrates and fats in the mullet from the values arrived at since protein yield 19·121 K J/1 O_2 (19·121 J/ml O_2) corrected value for ammonotelic animals (Brafield and Solomon 1972), the net caloric yield from protein is 13040·3 J kg^{-1} fish [i.e. 682·0 (ml O_2) × 19·121].

From non protein RQ value of 0·81 it appears that energy yield from carbohydrates and fats are 36·8 and 63·2 respectively, of the total yield of 20·125 J/ml O_2 (Dowban 1969). From this it can be estimated that the total yield from carbohydrate is 1378·2 J kg^{-1} and that of fats is 2367·3 J kg^{-1}. The anaerobic energy utilization from carbohydrate can be calculated from the amount of glycogen (glucose) utilized for the production of lactate.

The total lactate produced for 5 h = 9·64 m mol. Glycogen (glucose) utilized for 9·64 m mol of lactate production = 9·64/2 = 4·82 m mol.

The energy obtained from one mol of glucose through glycolysis is 52 Kcal which is equivalent to 217·57 K J (217·57 J/one m mol) (Dowban 1969). From this it can be estimated that the total yield from anaerobic source is 1048·5 J (i.e. 4·82 × 217·57 J). If the minor amount of energy derived anaerobically from protein is disregarded (the role of anaerobically product of NH_3 may be more to combat the acid-base imbalance caused by lactic acidosis rather than for obtaining energy), total energy utilized for the entire 5 h of mullet, exercised at 20 cm/s can be estimated as 17834·3 J kg^{-1} fish. The percentage of energy utilized by oxidative breakdown of proteins, carbohydrates and fats in mullet, exercised at 20 cm s^{-1} are more than 73·1, 7·7, 13·3 respectively and the remaining percentage of 5·9 is derived from anaerobic source.

The NP RQ derived likewise for mullet exercised at 77 cm s^{-1} is 0·6, which is quite below the conventional RQ values for fat. Therefore it is assumed that non-protein substrate is only fat and glucose is not involved in oxidative energy release. With this assumption the amounts of energy derived by mullet exercised at higher speed (77 cm/s) are estimated as 33587·9, 2841·3, 1305·4 J kg^{-1} fish from proteins, fats and anaerobic source respectively, being 89·0, 7·5, 3·5% of a total of 37734·7 J kg^{-1}/kg fish. It appears that the difference in energy utilization at 20 and 77 cm/s is mainly in the lesser use of carbohydrates and greater use of proteins at 77 cm/s. The lower NP RQ may also be due to the over estimation of the maximum possible aerobic NQ. Eventhough the percentage of anaerobic energy utilization at 77 cm/s is less, the absolute energy derived from anaerobic source is higher than that at 20 cm/s. It appears that anaerobic energy utilization is higher during the initial phase of exercise and the percentage of anaerobic energy during the 1st h of swimming is likely to be 25–30% of total energy utilized at that time and indeed during steady swimming anaerobic is not final. To arrive at more precise energy estimations only one means of energy derivation from the available data and the validity of the assumptions have to be checked further. But it is felt that this method of approach would be of some help in elucidating the problem of energy budgeting in fish.

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Wing microsculpturing in two Arabian species of termite genus *Amitermes* (Termitidae, Amitermitinae)

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Abstract. To understand the intra-generic variability of wing microsculpturing in *Amitermes* (hitherto known in a single species, *Amitermes belli*), two other species from Arabia (*Amitermes messinae* and *Amitermes* sp.) were studied. Microsculpturing consists of two elements—papillae and micrasters. There is an intra-generic range of complexity in micrasters, from the simple nonasteroids to the complex asteroids. The general pattern conforms to the condition in the subfamily Amitermitinae as a whole.

Keywords. Wing microsculpturing; *Amitermes*; Isoptera; termites.

1. Introduction

Wing microsculpturing in termites (Isoptera) has been studied extensively under both the light and the scanning electron microscopes by Roonwal and co-workers (Roonwal 1983, 1985a, b, 1986; Roonwal and Chhotani 1967; Roonwal and Rathore 1977, 1982, 1985; Roonwal and Verma 1980, 1983; Roonwal *et al* 1974). A dense and elaborate carpet of microstructures (size ca. 0·5–24 μm) occurs on both surfaces of wings. Its evolutionary, phylogenetic and systematic significance has also been discussed (Roonwal 1983, 1985a, b, 1986).

Several genera of the subfamily Amitermitinae (family Termitidae) have also been studied and intra-generic and inter-generic differences shown to occur (Roonwal 1983, 1985a; Roonwal and Rathore 1977, 1982, 1985; Roonwal and Verma 1980, 1983; Roonwal *et al* 1974). But the widespread genus *Amitermes* Silvestri has been studied inadequately, with only a single species, *Amitermes belli* (Desneux) from India examined. With two more species from Arabia, now available, we are in a much better position to examine intra-generic variability in microsculpturing.

2. Material and methods

Winged imagoes of both the species studied, viz. *Amitermes messinae* Fuller and *Amitermes* sp., were obtained from ground swarming holes near Muscat (Sultanate of Oman, at the south-eastern end of the Arabian Peninsula) in February 1986. Wings were mounted in glycerine (this gives a much better definition than Canada balsam mounts) and studied under the light microscope.

3. Results

3.1 *A. messinae*

Wings small, transparent, pale brown. Hairs common on the margins, fewer and

smaller on the membrane; length: upper margin 40–110 μm, lower margin 50–70 μm, on membrane 30–40 μm (figures 1 and 2).

3.1a *Microsculpturing*: Consists on both the upper and lower wing surfaces of two

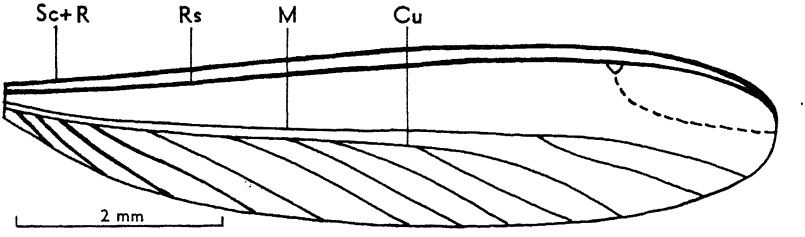

Figure 1. *A. messinae* outline of right forewing, to show venation. [Cu, Cubitus; M, media; Rs, radial sector (note the extra branch); Sc + R, subcosta + radius].

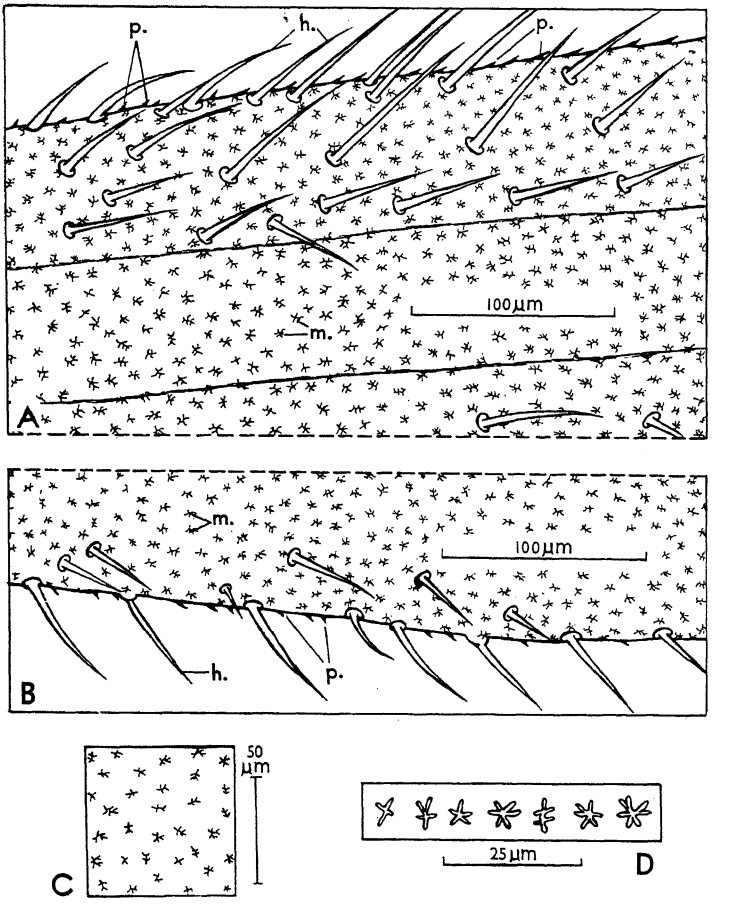

Figure 2. *A. messinae* portions of right forewing in middle part, to show microsculpturing. **A.** Upper edge. **B.** Lower edge. **C.** Middle of wing membrane, to show micrasters. **D.** Micrasters enlarged and rearranged. (h, Hairs; m, micrasters; p, papillae).

elements, viz. papillae and micrasters. (i) Papillae: Small (length 3–5 μm), pointed and thorny. In a single row on the upper and lower wing margins, none on the membrane; larger on the upper margin than on the lower. (ii) Micrasters: A dense carpet (density 7200/mm^2) (size 6–7 μm × 6–8 μm) of various types, both asteroid and nonasteroid (with 4–7 arms) present all over wing.

3.2 *Amitermes sp.*

Wings and hairs as in *A. messinae*. Length of hairs on upper wing margin 50–110 μm, on lower margin 40–70 μm and on membrane 30–40 μm (figures 3 and 4).

3.2a *Microsculpturing*: As in *A. messinae*. (i) Papillae: Small (length 2–4 μm), pointed and thorny. Present as a single row at the upper margin of wing; absent on the lower margin. (ii) Micrasters: As in *A. messinae*, but only the simple types, the nonasteroids, present with 1–5 arms.

4. Discussion

With wing microsculpturing now known in 3 species of *Amitermes*, we can have a fairly good idea of intra-generic variation. In all the 3 species, only two types of elements occur, viz. papillae and micrasters. The papillae are always small, pointed and thorny and occur in a single row on the wing margins (figure 5) in *A. messinae* on both the upper and lower margins, and in *A. belli* and *Amitermes* sp. on the upper margin only.

The micrasters display considerable range of diversity, the increasing complexity being in the following order (figure 1):

Amitermes sp.→*A. belli*→*A. messinae.*

In *Amitermes* sp. only the simple, nonasteroid micraster, with 1–5 arms, occur, while in *A. belli* both asteroids and nonasteroids, with 5–7 arms, occur. The same condition as in *A. belli* is found in *A. messinae*, but the range of nonasteroids is slightly greater and 4-armed micrasters also occur in addition. Furthermore, the micrasters are of the thin, stringy type in *A. belli* and thick in the other two species.

Thus, the condition in *Amitermes* is consistent with that in the subfamily Amitermitinae as a whole (Roonwal 1983), and the relative primitiveness of this subfamily within the family Termitidae (the higher Termites) is confirmed.

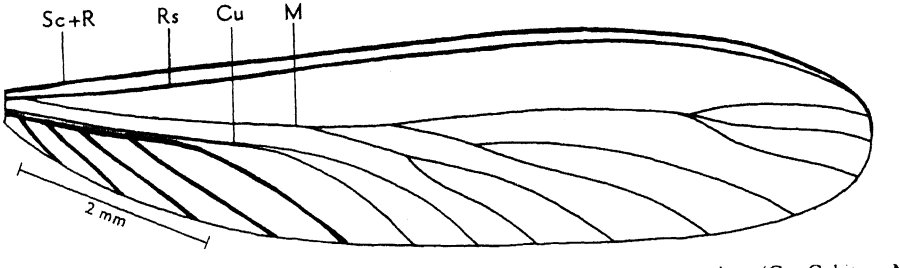

Figure 3. *Amitermes* sp. outline of right hindwing, to show venation. (Cu, Cubitus; M, media; Rs, radial sector; Sc+R, subcosta+radius).

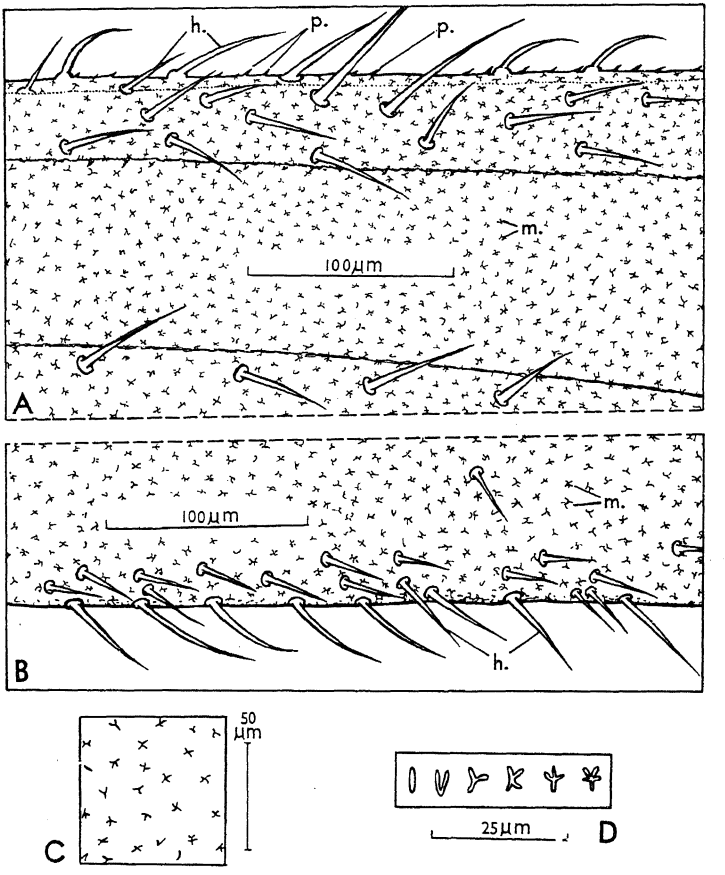

Figure 4. *Amitermes* sp. portions of right hindwing in middle part, to show microsculpturing. **A.** Upper edge. **B.** Lower edge. **C.** Middle of wing membrane, to show micrasters. **D.** Micrasters enlarged and rearranged. (h, Hairs; m, micrasters; p, papillae).

AMITERMES	NONASTEROID						ASTEROID			
	I	II	III	IV	V	VI	VII	VIII	IX	X
A. BELLI										
A. MESSINAE										
A. SP.										

Figure 5. Variation in the complexity of micrasters on the wings of 3 species of *Amitermes* (*A. belli*, *A. messinae* and *Amitermes* sp.).

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Haematological effects of sublethal concentration of formalin on *Sarotherodon mossambicus* (Peters)

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Abstract. Exposure of fish *Sarotherodon mossambicus* (Peters) for 24 h to 80 ppm formalin induced lowering of total erythrocyte count and increase in haemoglobin and haematocrit. Erythrocyte constants and indices of exposed fish were higher than of the control fishes. The sum total of the effect of sublethal concentration of formalin on the peripheral blood of *Sarotherodon mossambicus* was macrocytosis and hyperchromia.

Keywords. Formalin toxicity; haematology; *Sarotherodon mossambicus*.

1. Introduction

Despite the fact that the toxic effects of several chemicals employed in fish disease therapy have been analysed by various workers, there seems to be little attention given to formaldehyde—an effective chemotherapeutic agent successfully employed against several infectious conditions, particularly those affecting the body surface/skin of fishes (Schnick 1974; Roberts and Shepherd 1979). The only publications that have given attention to formaldehyde poisoning of fishes are those by Wedemeyer (1971), Smith and Piper (1972) and Williams and Wootten (1981). In this paper the results of the study on the effects of sublethal concentration of formalin on the peripheral blood of *Sarotherodon mossambicus* (Peters) are presented.

2. Materials and methods

Specimens of *S. mossambicus* collected from a local freshwater pond, were transported alive to the laboratory in large polythene buckets. Fishes were acclimatized for one day to laboratory conditions by keeping them in large aquarium tanks containing filtered well-water.

Apparently healthy fishes ranging from 9·5–11 cm in standard length were selected for the present study. Ten fishes were maintained as control and 10 others were used for experimental purpose.

For TLm determination and for experimental studies, glass troughs of size 30 cm diameter, 15 cm height and a capacity of 6 L were used. Filtered well-water (mean temp. $27 \pm 1°C$; pH 7·9) was used throughout the experiment. TLm concentration (24 h) was determined by adopting the procedure suggested by Doudoroff *et al* (1951) for sewage and industrial wastes.

Haematological analyses were carried out employing standard techniques (Hesser 1960; Blaxhall and Daisley 1973). Blood samples were collected by severing the caudal peduncle. A 3 : 2 mixture of ammonium oxalate and potassium oxalate at the rate of 0·5–1 mg/ml blood was used as anticoagulant.

Total erythrocyte count (TEC), haemoglobin content (Hb) and haematocrit (Ht) were determined and from these results, the erythrocyte constants [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] and the erythrocyte indices [volume index (VI), colour index (CI) and saturation index (SI)] were computed. The results were statistically tested by the Student's '*t*' test.

3. Results

In tables 1 and 2 the results of haematological analyses of the control and experimental fish respectively are presented. Table 3 shows the results of Student's '*t*' test comparing the haematological parameters of control and experimental fish.

3.1 *TEC* ($\times 10^6/mm^3$)

TEC of the control fishes ranged from 2·22–2·56 and of the experimental from 1·75–2·65. The mean counts for the two groups were 2·35 and 2·14 respectively. The results indicate that exposure to formalin reduced the erythrocyte count of the fish. From the results of the '*t*' test it may be seen that the observed difference in TEC between the control and experimental fish is statistically significant ($P < 0{\cdot}05$).

3.2 *Hb* (g%)

For the control fish Hb content recorded a mean value of 11·19 with a range from 9·2–11·8. For the experimental fish it recorded a significantly higher mean value of 15·08 ($P < 0{\cdot}01$); the range of Hb content here was from 13·8–16·2. The result shows that exposure to formalin had an advantageous effect over Hb content of the fish.

3.3 *Ht* (%)

In the control fish Ht ranged from 24·27–35·37 with a mean of 28·63. In the

Table 1. Haematological parameters of 10 control specimens of *S. mossambicus*.

TEC ($\times 10^6/mm^3$)	Hb (g%)	Ht (%)	MCV (μ^3)	MCH (pg)	MCHC (%)
2·56	11·4	28·73	112·23	44·53	39·68
2·45	11·0	30·00	122·45	44·90	36·67
2·27	11·8	26·67	117·49	51·98	44·24
2·49	11·8	28·96	116·30	47·79	41·09
2·39	11·8	24·27	101·55	49·37	48·62
2·26	10·6	32·58	144·16	46·90	32·54
2·23	9·2	24·73	110·90	41·26	37·20
2·30	11·0	35·37	153·78	47·83	31·10
2·28	11·4	25·72	112·81	50·00	44·32
2·22	11·8	29·28	131·89	53·15	40·30
Mean ± SE.					
2·35 ± 0·04	11·18 ± 0·25	28·63 ± 1·05	122·36 ± 4·87	47·77 ± 1·08	39·58 ± 1·63

Table 2. Haematological parameters of 10 fish (*S. mossambicus*) exposed to sublethal concentration of formalin.

TEC ($\times 10^6$/mm^3)	Hb (g%)	Ht (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	VI	CI	SI
2·65	15·2	31·0	116·98	57·36	49·03	0·96	1·21	1·24
2·44	14·8	29·0	118·85	60·66	51·03	0·97	1·27	1·29
2·34	14·8	31·0	133·48	63·25	47·74	1·08	1·33	1·21
1·83	13·8	28·0	153·01	75·41	43·28	1·25	1·58	1·10
1·80	14·0	26·0	144·44	77·78	53·85	1·18	1·63	1·36
1·75	16·0	29·0	165·71	91·43	55·17	1·35	1·92	1·39
1·90	16·2	34·0	178·95	85·26	47·65	1·46	1·79	1·20
2·20	15·8	32·0	145·45	71·82	49·38	1·19	1·51	1·25
2·12	15·4	28·0	132·08	72·64	55·00	1·08	1·53	1·39
2·34	14·8	32·0	136·75	63·24	46·25	1·12	1·33	1·17
SEM ± SE								
2·14 ± 0·09	15·08 ± 0·24	30·0 ± 0·72	142·57 ± 5·85	71·89 ± 3·31	49·84 ± 1·18	1·16 ± 0·10	1·51 ± 0·07	1·26 ± 0·03

Table 3. '*t*' values comparing haematological parameters of control and formalin exposed *S. mossambicus*.

Haematological parameters	'*t*' values
TEC	2·0087[a]
Hb	10·7137[b]
Ht	1·0205
MCV	2·2667[a]
MCH	6·5754[b]
MCHC	4·8503[b]
VI	2·7735[a]
CI	8·8230[b]
SI	8·4602[b]

[a] $P < 0·05$; [b] $P < 0·01$.

experimental fish mean Ht was slightly higher (30·0) than in the control. However, the difference in Ht between control and experimental fish was not statistically significant.

3.4 *Erythrocyte constants*

MCV of the experimental fish was 142·57 μ^3 compared to 122·36 μ^3 for the control fish. The difference in MCV between the control and experimental fish was statistically significant ($P < 0·05$).

MCH was also significantly altered by exposure to formalin. In the control fish MCH recorded an average of 47·77 pg whereas in the experimental fish it was much higher, with an average of 71·89 pg. The observed difference in MCH between the control and experimental fishes was significant at 1% level.

MCHC recorded very high value in formalin exposed fishes; the mean MCHC for this group was 49·84% in comparison with 39·58% for the control group. Here the difference between the control and experimental groups was statistically highly significant ($P < 0·01$).

3.5 *Erythrocyte indices*

In conformity with the increased MCV, VI of the experimental fish recorded an average value of 1·16. This clearly indicates that exposure to formalin induced swelling of the erythrocytes of the fish.

An increase in Hb content and MCH has been induced by formalin, consequently the CI of the experimental fish also recorded a considerably high mean value of 1·51 and this, along with the high SI (1·26), indicate that exposure to formalin induced hyperchromia.

4. Discussion

Wedemeyer (1971) observed pituitary activation in rainbow trout treated with 200 ppm formalin. Smith and Piper (1972) reported an increase in Ht and TEC in formalin treated rainbow trout. Williams and Wootten (1981) found that immediately after exposure to 200 ppm of formalin, Ht and Hb of rainbow trout significantly increased.

Comparing the results of the present study on *S. mossambicus* with the foregoing, a much lower concentration of formalin (85 ppm) itself was found to be lethal to the fishes. As for the effects, the haematological parameters of exposed fish were characterised by a decrease in TEC and an increase in Hb and Ht. Smith and Piper (1972) and Williams and Wootten (1981) also recorded increase in Hb and Ht in rainbow trout exposed to formalin. However, unlike the results obtained by the former workers for TEC, during the present study TEC was significantly lowered by formalin treatment. It may be noted that the mean corpuscular volume of formalin treated *S. mossambicus* was much higher than that of the control fish. This naturally would result in the reduction of the number of erythrocytes in a unit volume of blood. From the results, it would seem that it is the excessive increase in erythrocyte volume that apparently produced a picture of reduced TEC. It is, therefore, reasonable to conclude that the direct effect of formalin has been on the erythrocyte volume, not on its numerical abundance.

Williams and Wootten (1981) found that, even though immediately after exposure to formalin Hb and Ht of rainbow trout increased significantly, after 24 h exposure both parameters tended to return to normalcy. The present results on *S. mossambicus* are in disagreement with those of Williams and Wootten (1981) as the significant increase in Hb and Ht persisted even after 24 h exposure to formalin.

Increased Hb may be looked upon as a sort of compensatory reaction. Wedemeyer (1971) explained the increased pituitary activity in formalin treated rainbow trout on the basis of the chemical adversely affecting gill function. Such an interference with gill function can be expected to reduce its respiratory efficiency. It may be to compensate for this impaired respiratory efficiency that the Hb was increased in the treated fish. This sort of compensatory reaction is known to occur in fishes infected by certain parasites (Kabata 1970).

Exposure to formalin was found to result in an increase, though not statistically significant, in the Ht of *S. mossambicus*. Since the TEC of the experimental fish recorded a lower value than that of the control fish, presumably the increased Ht is the result of the increased MCV. Smith and Piper (1972) and Williams and Wootten (1981) also found an increase in the Ht of rainbow trout following exposure to

formalin. In the former study, since TEC also was higher, increase in Ht might have been due to increased number of blood cells; but in the latter study the cause of increase in Ht is not deducible.

Direct comparison of the results on erythrocyte constants and indices obtained during the present study with other like observations is not possible owing to lack of similar data from elsewhere. The results indicate that the erythrocytes of formalin exposed fish became hyperchromic (high CI) and macrocytic (high VI). The observed high CI is obviously not because of supersaturation of erythrocytes with Hb but because of the increased cell volume; the larger cells have accumulated more Hb (compared with the normal erythrocytes) resulting both in increased Hb and MCH. The overall toxic effect of sublethal concentration of formalin on *S. mossambicus* as indicated by the present study may be summarised as macrocytosis and hyperchromia.

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